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SOME EFFECTS OF A HARMFUL ORGANIC SOIL CONSTITUENT[1]

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(WITH ELEVEN FIGURES)

The investigation of infertile soils from various parts of the United States has received considerable attention in the last few years, and has been conducted along several converging lines in these laboratories. Among these is a thorough inquiry into the nature of the organic matter of soils. The results of these researches into the chemistry of the organic matter of the soil, its origin, transformation, and properties, have been reported upon to a large extent in a former bulletin of this Bureau and in scientific journals.[2] Several bodies have been isolated from such soils, which have quite different chemical properties, thus showing that there

[1] Published by permission of the Secretary of Agriculture.

[2] SCHREINER, O., and SHOREY, E. C., The isolation of dihydroxystearic acid from soils. Jour. Amer. Chem. Soc. **30**:1599. 1908.

———, The isolation of picoline carboxylic acid from soils and its relation to soil fertility. Jour. Amer. Chem. Soc. **30**:1295. 1908.

———, The presence of a cholesterol substance in soils; agrosterol. Jour. Amer. Chem. Soc. **31**:116. 1909.

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———, Pentosans in soil. Science N.S. **31**:308. 1910.

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———, The presence of secondary decomposition products of proteids in soils. Proc. Amer. Soc. Biol. Chem. **1**:47. 1907.

See also Bull. 53, Bureau of Soils, U.S. Dept. Agric.

SCHREINER, O., and SULLIVAN, M. X., Soil fatigue caused by organic compounds. Jour. Biol. Chem. **6**:39. 1909.

is a difference in the nature of the chemical bodies in different soils. Some of the bodies isolated contained nitrogen, while others free from this element contained carbon, hydrogen, and oxygen only. The presence of sulfur and phosphorus compounds has also been strongly indicated by these studies, although the actual identification of bodies of such nature has not been definitely accomplished. The results are sufficient, however, to show that a wide range of bodies of different composition exists in soils, and a more detailed knowledge of their nature and properties seems imperative in order to know and understand the nature of the material influencing crops in the field and the action of fertilizers upon these, either directly or indirectly, through the medium of root oxidation and reduction or by microbiologic and enzymotic influences.

In an earlier paper[3] it was demonstrated that the roots of plants possessed a very appreciable oxidizing power. This oxidizing power of the normal root was found to be influenced by the medium in which it grew. Thus good soils and their aqueous extracts promoted oxidation, while the poorer soils hindered this function of the roots. Some substances harmful to plant growth were also found to have an inhibitive effect on this oxidation. When smaller amounts were present, however, it was found that the oxidizing power of the roots was able to overcome the harmful influence. An examination in one case, namely that of vanillin, where it was possible to demonstrate by colorimetric test the presence or absence of minute amounts, showed that this disappeared entirely from solution under the influence of the oxidizing power of the roots. It was further shown that the fertilizer salts, in addition to promoting plant growth, also had a very strong influence in promoting this enzymotic effect, this being especially marked in the case of sodium nitrate and lime.[4] The action of fertilizer salts and the influence of such harmful soil constituents were further investigated, therefore, and the results given in connection with the

[3] SCHREINER, O., and REED, H. S., Studies on the oxidizing power of roots. BOT. GAZETTE **47**:355. 1909. See also Bull. 56, Bureau of Soils, U. S. Dept. Agric. 1909.

[4] SCHREINER, O., and REED, H. S., The power of sodium nitrate and calcium carbonate to decrease toxicity in conjunction with plants growing in solution cultures Jour. Amer. Chem. Soc. **30**:85. 1908.

other factors reported in this paper. All of the harmful bodies which had been studied and presented in previous papers had a distinct influence on the roots, causing them in many cases to become stunted or swollen and darkened at the tips, or to show other physiological irregularities of the same kind as is exhibited by the roots in different extracts from infertile soils. It is, therefore, of interest to know further the influence which such altered root conditions would have upon the composition of the soil solution and the influence of added fertilizers.

One of the bodies isolated from a number of unproductive soils is a definite crystalline body identified as dihydroxystearic acid melting at 98°–99° C. It can be prepared by the oxidation of elaidic acid in the laboratory. In these experiments the dihydroxystearic acid used had been prepared in this manner. The frequent occurrence of this body in such soils and its disappearance therefrom by processes which promote aeration or oxidation made this especially suitable for further study of its effects on plant development in relation to the concentrations of soil solutions or fertilizer application.

The dihydroxystearic acid can be isolated from a soil containing it by treatment with a 2 per cent sodium hydroxid solution, and, after allowing the mineral material to settle, the alkaline extract is separated and made acid with a slight excess of acetic acid. The so-called humus precipitate which is thus formed is filtered off and the clear filtrate is shaken out with ether and the ether solution allowed to evaporate on the surface of a small quantity of water. The dihydroxystearic acid is left on the surface of the water, together with other impurities extracted by the ether. The impurities can be largely removed by heating the water to boiling, and filtering. Fig. 1 shows the effect of a solution of this nature on wheat seedlings when the material is dissolved in much water. The dihydroxystearic acid, when dissolved in a small volume of water and then cooled, crystallizes out in the form of small plates or leaflets arranged in radiating clusters. Fig. 2 shows the effect of the purified substance in various concentrations. The details of the method of isolating and purifying will be found in the paper cited.

Dihydroxystearic acid can also be prepared in the laboratory by starting with oleic acid, which by treatment with nitrous oxid is changed to the isomeric elaidic acid. The elaidic acid thus formed is dissolved in a solution of potassium hydroxid and oxidized by a solution of potassium permanganate, one of the products under suitable conditions being dihydroxystearic acid.

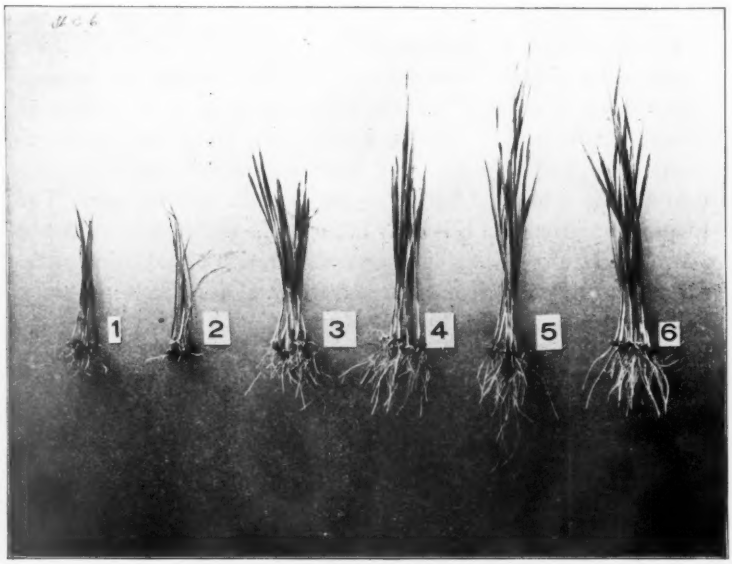

FIG. 1.—Wheat seedlings grown in extract obtained in the method for isolating dihydroxystearic acid from soils: *1*, *2*, undiluted extract; *3*, *4*, one part of extract, one part of distilled water; *5*, *6*, one part of extract, nine parts of distilled water.

Care has to be taken that the oxidation does not proceed too far, for the dihydroxystearic acid, as already mentioned above, is very readily oxidized to other compounds.

In this paper are reported experiments on the effect of a harmful soil constituent upon plant growth and upon soil solutions and fertilizer action, especially with reference to the ratio of phosphate, nitrate, and potash originally present and removed by wheat seedlings in the course of the experiment.

Solution cultures containing the three fertilizer ingredients, namely P_2O_5, NH_3, and K_2O, as calcium acid phosphate, sodium nitrate, and potassium sulfate, respectively, in all possible ratios of one, two, and three constituents, varying them in stages of 10 per cent, were prepared, the concentration being 80 parts per million in these constituents. In a similar set of cultures there

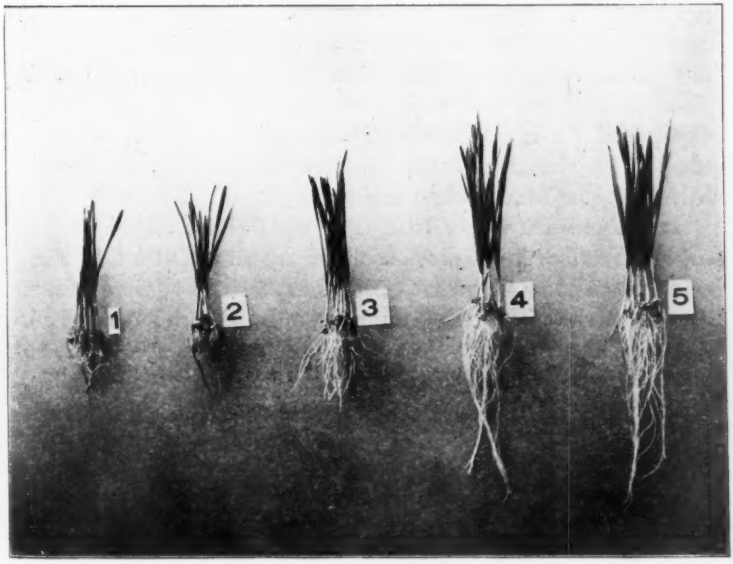

FIG. 2.—Wheat seedlings grown in solutions of dihydroxystearic acid from soils: *1*, solution of dihydroxystearic acid, 200 parts per million; *2*, 100 parts; *3*, 50 parts; *4*, 20 parts; *5*, control in distilled water.

was added 50 parts per million of dihydroxystearic acid to each culture. The selection of the salts as carriers of the phosphate, nitrate, and potash, and the terms P_2O_5, NH_3, and K_2O are in harmony with the practice in fertilizer work. The salts selected, it will be seen, are also carriers of calcium, of sodium, and of sulfate, and the three salts, therefore, were practically the best that could be selected for giving at the same time other needed constituents. The details of this method of experimentation have been given in

an earlier paper, and the reader is referred to this for a full explanation of the use of the triangular diagram and the results obtained by growing seedling wheat in these various culture solutions without the presence of any added harmful substance.[5] Wheat seedlings were then grown in these various cultures and observations made in regard to general development, the effect on the root growth and appearance, and on root oxidation, and at the termination of the experiment the green weight of the plants was taken. The solutions were changed every three days and an analysis made, the phosphate, nitrate, and potash being determined, thus giving the concentration of these elements and their ratios existing at the end of every three-day period for comparison with the original concentration and ratio. This changing of the solutions was kept up for twenty-four days, thus making eight changes.

In the present work a triangle of solution cultures similar to the one described in the previous paper was set up, with the difference that 50 parts per million of dihydroxystearic acid were present in each culture. This set of cultures grew from April 2 to April 26. The set without the dihydroxystearic acid grew from February 26 to March 21. While these two sets are not strictly comparable, owing to the fact that they were grown at different times, though under very similar greenhouse conditions and for the same length of time as well as in the same time of year, one closely following the other, the results show, nevertheless, very strikingly the effect of dihydroxystearic acid, and are duplicated or substantiated by two further experiments in which the sets were grown simultaneously.

The analytical results, however, were not so complete in these later tests, owing to the inability to handle the 396 separate determinations necessary every three days. The results given in this paper, therefore, are largely based on the first set, for the reason that the analytical results were more complete than in the other sets mentioned.

The difference between the cultures containing the dihydroxystearic acid and those without this substance was very marked,

[5] SCHREINER, O., and SKINNER, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth. BOT. GAZETTE 50:1. 1910.

and was especially striking in those sets where both triangles of cultures were grown simultaneously. In addition to the general appearance of the tops, the presence of this harmful body produces still other effects readily recognized by the investigator, and forming on the whole a better physiological indicator of the toxicity than the growth of tops, as has been well recognized by physiologists generally in conducting similar work. Reference is here made to the action of the body on root condition and growth. The root being bathed by the solution, and being, moreover, the delicate mechanism of absorption, is often a more sensitive indicator than is the growth of the top. The most marked effects of dihydroxystearic acid on roots are strongly to inhibit their growth and to produce enlarged or swollen tips, which are frequently very dark in color and often turned back in the form of hooks. These phenomena are observed even when the injury is not so great as to kill the plants. This action of the body on the roots is influenced by the conditions of growth imposed upon the plant by the different fertilizer ratios. This is shown by the general notes taken on one of these sets one week after growth in the cultures had begun.

All the plants in the solutions having no nitrogen appear to be dying. The roots are already dead, have made little growth, and are much discolored, the tips are dark and swollen and a large number turned upward. The tops have made some growth, some of the leaves are curled, but most are still fresh and green.

The plants in the solutions containing no phosphate are similar to the above; the roots and tops are somewhat better, but still show an undoubted harmful action, the roots being dark and flimsy, tips swollen and often turned upward, the greater number being dead.

The plants in the solutions on the potash base line are better than the two sets described above. The upper parts of the roots of these plants are dark, the lower sections that have recently grown out are white and clear, but the tips are still dark and swollen, and many are turned upward.

The plants in the interior of the triangle, where all three fertilizer ingredients are present, are fairly good, with no great difference noticeable at this stage, but showing somewhat better plants near

the center. The roots, on the other hand, are already showing differences. They are rather poor along the line having 8 parts per million of NH_3. The upper and older part of the roots is dark, the newer portion is white and clear, but the tips are swollen. The general condition of the roots, as a whole, is a great deal better than in case of those in the boundary lines of the triangle. The condition of the roots on the line having 8 parts per million P_2O_5, and on the line having 8 parts per million K_2O, are about the same as on the 8 parts per million NH_3 line.

Farther within the triangle the general condition of the roots is much better. The solutions in cultures number 25, 32, 33, 34, 42, and 43 show by their roots that the harmful effect of the substance has been somewhat overcome: they are lighter than the others, in fact most of the roots, except the upper and older sections, are white and clear, but the tips are still slightly swollen and somewhat bent.

Another property of the roots which is influenced by harmful bodies is that of root oxidation. It has been shown that plant roots possess a very appreciable power of oxidation, and that this power is stronger in good soils than in poor, or in their extracts, and that harmful bodies retard this oxidation, and beneficial bodies augment it. Fertilizer salts were shown to increase root oxidation, and through this action a reduction in the quantity of the harmful body present was produced.

Some further observations were made in connection with the present investigation in regard to oxidation and the effect of dihydroxystearic acid upon this function. The effect was so marked in the concentrations used, namely 50 parts per million, that the roots at the end of the experiment were found to be almost wholly lacking in ability to oxidize aloin used as an indicator in the manner described in the publication referred to above. Some oxidizing power was still possessed by the cultures in the interior of the triangle, where, as mentioned above, the growth was better than in other regions, although even here the power of oxidation was greatly impaired. Nevertheless, it is interesting to note that the condition which produced the best general development of the plants is closely associated with a power of root oxidation.

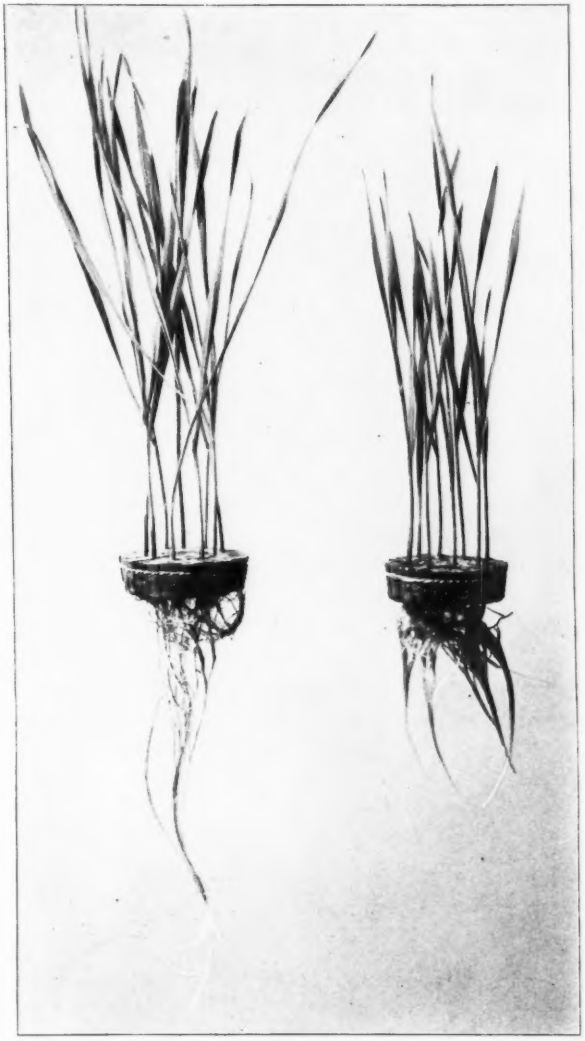

FIG. 3.—Wheat plants growing in a culture solution containing a fertilizer mixture composed of phosphate 60 per cent, nitrogen 20 per cent, potash 20 per cent: *1*, without dihydroxystearic acid; *2*, with dihydroxystearic acid.

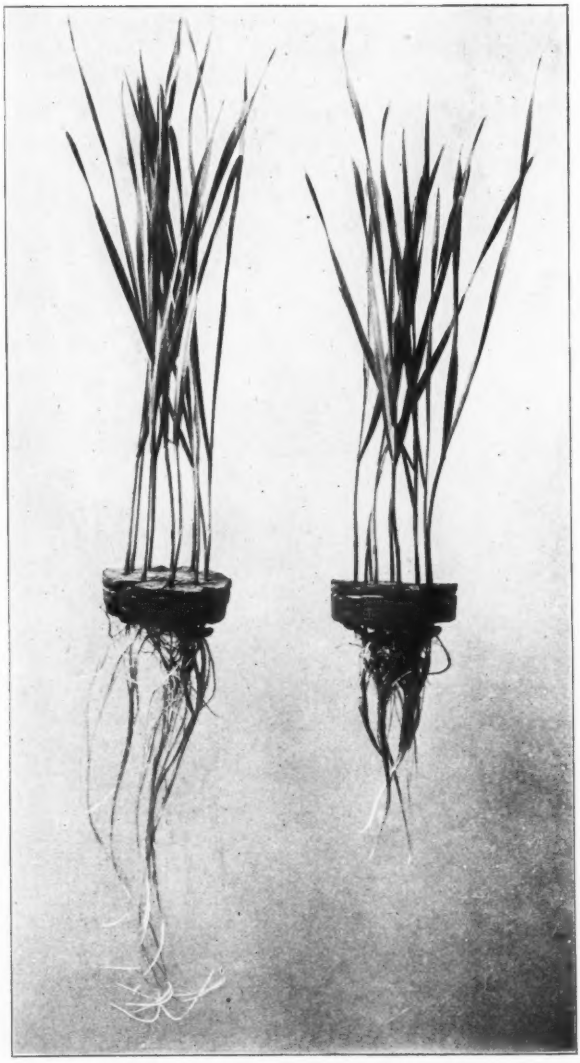

FIG. 4.—Wheat plants growing in a culture solution containing a fertilizer mixture composed of phosphate 20 per cent, nitrogen 60 per cent, potash 20 per cent: *1*, without dihydroxystearic acid; *2*, with dihydroxystearic acid.

Lower concentrations of dihydroxystearic acid would doubtless be better suited for a more thorough study of this matter.

Some of the plants grown in solutions with and without dihydroxystearic acid are shown in figs. 3, 4, and 5. The cultures taken represent a fertilizer rich in phosphoric acid, one rich in nitrate, and one rich in potash. The results shown in these photographs are representative of the general effect over all the region of the triangle from which they are taken.

GREEN WEIGHT OBTAINED IN THE VARIOUS CULTURES

The green weights obtained in the 66 cultures of the first set are given in the triangular diagram shown in fig. 6. It will be seen that, in harmony with the results given in the previous paper reporting the experiment without the harmful substance, the growth with the single elements or along the lines where mixtures of two occurred was in general less than within the triangle. The region of greatest growth in the solutions without the dihydroxystearic acid was approximately in the middle of the 10 and 20 per cent phosphate lines. In the dihydroxystearic acid cultures it might be said in general that this region of greater growth was displaced along these lines toward the nitrogen side.

The total growth made in the 66 cultures without the dihydroxystearic acid was 207 grams as against 114 grams in the case of the 66 cultures with the 50 parts per million of dihydroxystearic acid, or, putting the first as 100, the latter becomes 55. In other words, the plants with the dihydroxystearic acid made only, as an average of the 66 cultures, a growth of 55 per cent. It will be seen that the harmful substance produced its greatest effect in general along the periphery of the triangle, except, perhaps, along the phosphate-nitrogen line. In the interior of the triangle, where the greater growth occurred under the more normal conditions, the depression caused by the substance was not so marked, and this seems to be especially true in the region nearer to the nitrate end, in which, as already pointed out, the greater growth occurred in the cultures where the dihydroxystearic acid was present.

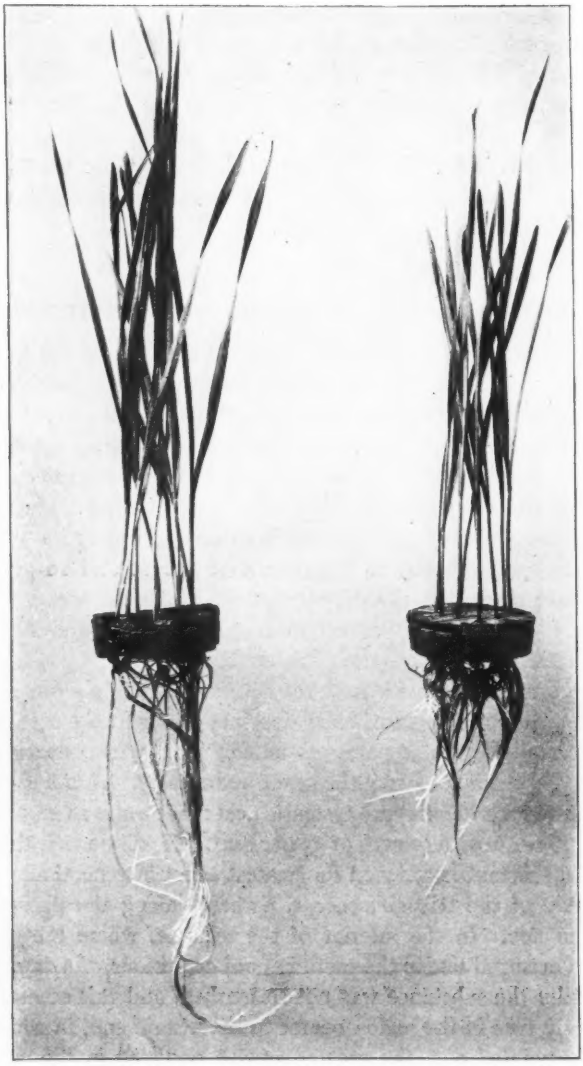

FIG. 5.—Wheat plants growing in a culture solution containing a fertilizer mixture composed of phosphate 20 per cent, nitrogen 20 per cent, potash 60 per cent: *1*, without dihydroxystearic acid; *2*, with dihydroxystearic acid.

CONCENTRATIONS OF $P_2O_5+NH_3+K_2O$ FOUND IN THE VARIOUS CULTURES

As has already been stated, the solutions were analyzed every third day for the three component fertilizer parts, phosphate, nitrate, and potassium, expressed as P_2O_5, NH_3, and K_2O. The original concentration in these elements was in the sum total 80

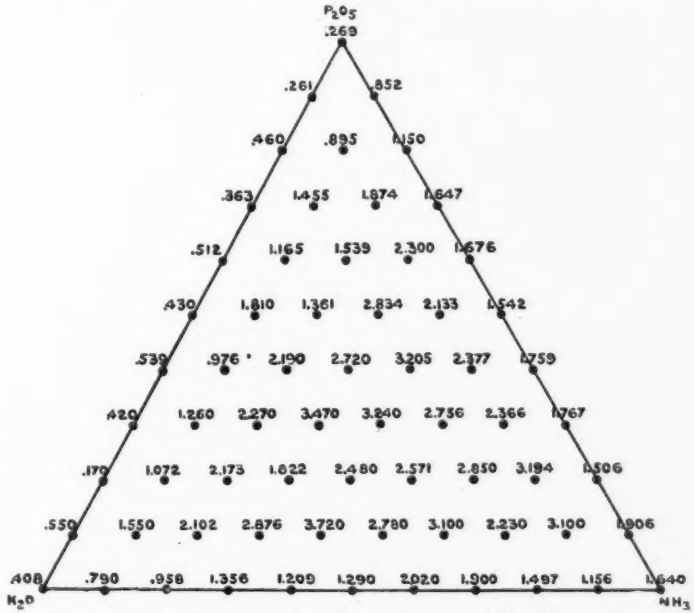

FIG. 6.—Green weight of wheat grown in 66 cultures with different proportions of P_2O_5, NH_3, and K_2O.

parts per million. After analysis the sum total of the three component parts was again calculated and the average concentration of these three elements was ascertained for the eight periods. These average concentrations will be found in the diagram in fig. 7. In practically all cases where dihydroxystearic acid was present, the concentration of the solution was not reduced as much as in the corresponding solution without dihydroxystearic acid, shown in fig. 4 of the former paper.

RATIOS OF P_2O_5, NH_3, AND K_2O FOUND IN THE VARIOUS CULTURES

In the diagram fig. 8 are given the original ratios of the fertilizer constituents, the ratios left in these solutions as shown by analysis, and the corresponding ratio of the removed constituents, as in the former paper. As before, the large dots in the diagram represent the original ratios according to the scheme previously

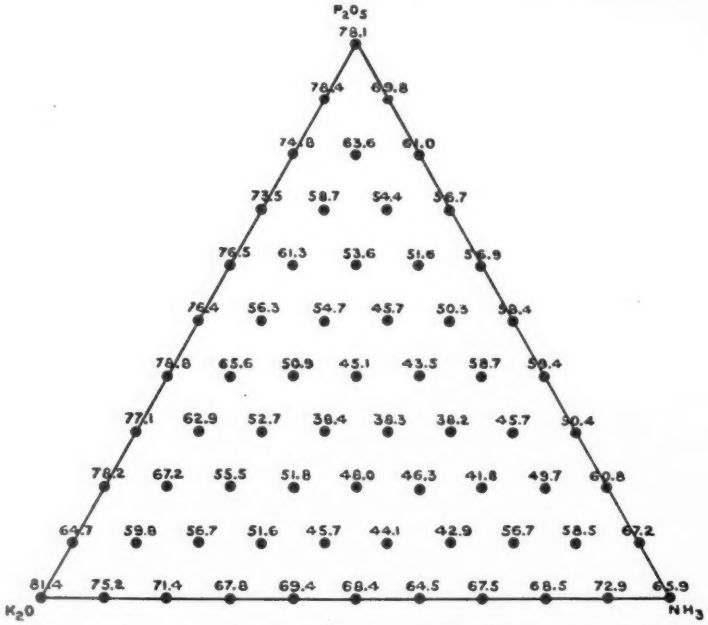

FIG. 7.—Average concentration in parts per million of P_2O_5 + NH_3 + K_2O of the solution after the growth of 10 wheat plants; concentration of original solution was 80 p.p.m.

explained. The circles indicate the ratio left in the solution as shown by analysis, and the other end of the line indicated by an arrow shows the corresponding ratio of the removed materials.

In the former experiment without dihydroxystearic acid there was a decided tendency for these lines to converge toward a region somewhat below the center; that is, the solutions near this central area changed least in their ratio, and the farther the ratios were

removed from this central area originally, the more were they altered in the course of the experiment. This area was between the 10 and 20 per cent phosphate line, and the area of greatest growth occurred in this same region. It is in this region of greatest growth, therefore, that the greater absorption of nutrients took place with the least change in ratio; in other words, the solutions repre-

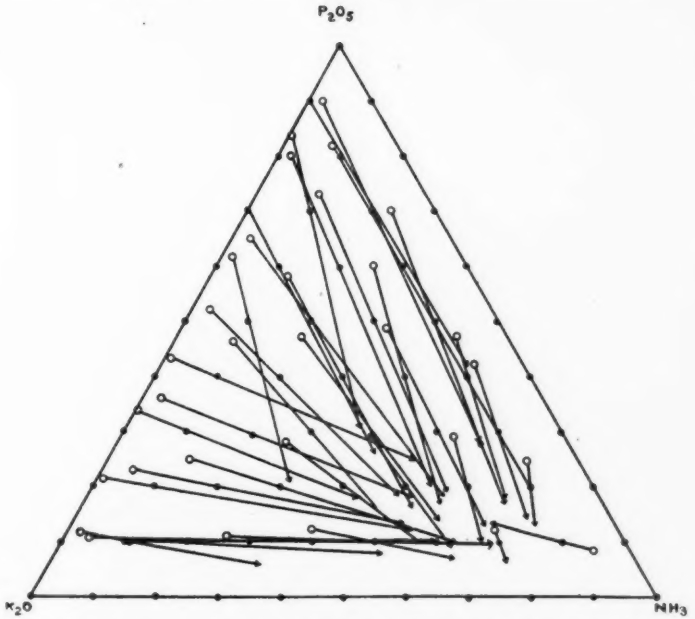

FIG. 8.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution; the dots indicate the ratio of the constituents in the original solution; the circles show the ratio of the constituents in the solution after growth; and the arrows show the ratio of the decrease.

sented by this region offered the best environment for plant development and the best ratios for the absorption of plant nutrients.

The diagram shown in fig. 8 for the experiment with dihydroxystearic acid shows on the whole a migration of the lines so that they point to an area somewhat nearer to the nitrate end of the triangle. It will be remembered from the data already discussed

that there is a tendency for the area of greatest growth under these conditions to be nearer this end.

In other words, when dihydroxystearic acid is present the results show a tendency for the ratios of the materials removed from the solution to fall nearer to the nitrogen end of the diagram than they do in the case of cultures where this substance is absent. This

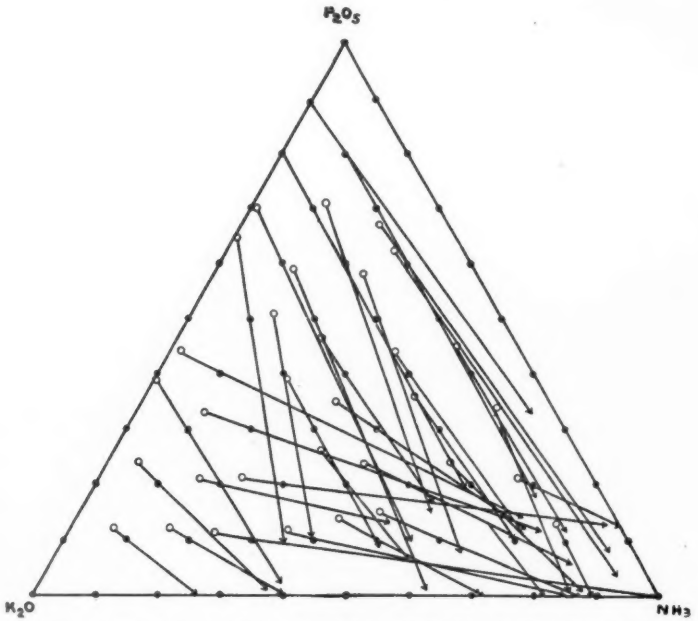

FIG. 9.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution, in the first period.

tendency is marked from the very first, as is shown by the diagram given in fig. 9 for the first period. Moreover, the points, for instance, of the 10 per cent mixture of P_2O_5 lie very low, and this tendency to lie lower than in the cultures which contained no dihydroxystearic acid is found throughout the experiment, though it is not equally marked in all periods. The average effect has already been given in the diagram fig. 8. The strong tendency

which is shown when this substance is present for a proportionately, and sometimes even absolutely, greater decrease in the nitrogen of the solutions, is strikingly shown in this first period (fig. 9). This varies somewhat from period to period; thus, for instance, in the fourth period, shown in fig. 10, the ratio change is fairly normal, though the usual tendency is seen again in the seventh period, a

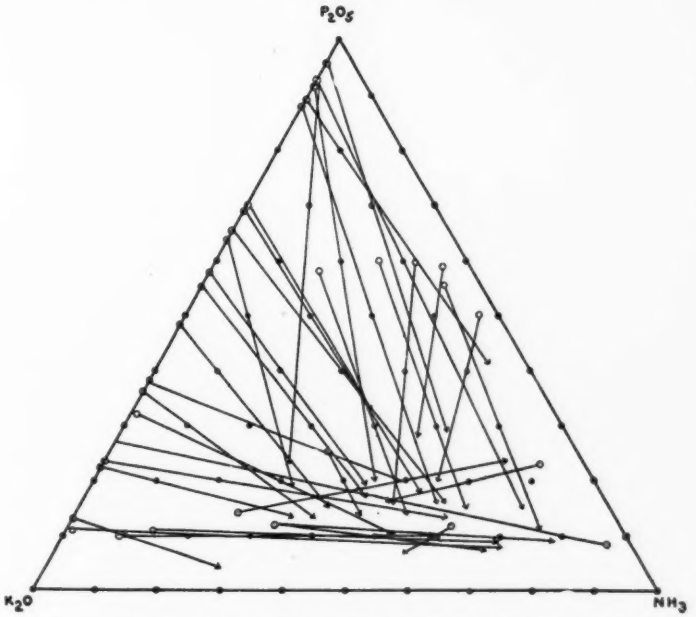

FIG. 10.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the solution, in the fourth period.

diagram of which is presented in fig. 11. These diagrams for the several periods illustrate rather well the general tendencies brought out by an examination of the analytical data. As a rule, beyond the second or perhaps the third period the diagrammatic representation of the result is on the whole uniform, but is influenced undoubtedly by the conditions of growth during any period; in other words, by weather and other conditions, which is shown perhaps quickest

in the nitrate removal from the cultures. The influence of light conditions on different days has already been discussed in the previous paper.

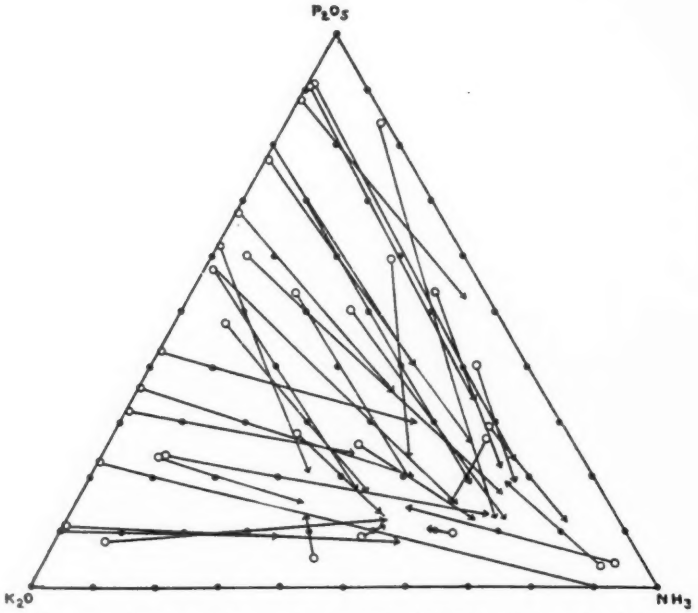

FIG. 11.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the solution, in the seventh period.

THE COMPARATIVE EFFECT PRODUCED ON THE DIHYDROXYSTEARIC ACID CULTURE SOLUTIONS BY THE FERTILIZER MIXTURES COMPOSED MAINLY OF P_2O_5, NH_3, AND K_2O RESPECTIVELY

In the experiment presented, as well as in two others in which both sets of cultures were grown simultaneously, there was noticed a more general tendency toward normal development of the plants in the presence of dihydroxystearic acid when the culture solutions contained all three of the fertilizer substances. It was further apparent that there seemed to be a tendency for more normal development in a region nearer to the nitrate end. In order to

trace out this matter, a comparison of the three experiments has been made and is hereby presented. In this way the solutions which contained in all cases 50 per cent or more P_2O_5 are considered in one group; all those which contained 50 per cent or more NH_3 in a second group; and those with 50 per cent or more K_2O in a third group. By grouping the cultures thus, an average result is obtained with the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers.

A comparison of the average green weight in each of these groups of cultures was made with those in the corresponding groups in the cultures where dihydroxystearic acid was absent. The relative growths thus obtained, taking the growth without the dihydroxystearic acid as 100, are contained in table I.

TABLE I

SHOWING THE AVERAGE RELATIVE GROWTH MADE IN THE GROUP OF SOLUTIONS WITH DIHYDROXYSTEARIC ACID AND CONTAINING FERTILIZER SALTS HAVING THE COMPOSITION OF 50 TO 100 PER CENT OF ANY ONE OF THE COMPONENTS P_2O_5, NH_3, AND K_2O.

EXPERIMENT	RELATIVE GREEN WEIGHT (Green weight without dihydroxystearic acid = 100)		
	P_2O_5 50–100 per cent	NH_3 50–100 per cent	K_2O 50–100 per cent
1	49	67	43
2	60	62	62
3	71	68	61

In the first column is given the number of the experiment; in the second column the average relative green weight obtained in the solutions having mainly phosphatic fertilizers; in the third column the same result for the cultures of mainly nitrogenous fertilizers; and in the fourth column the results obtained when mainly potassic fertilizers were present. The results indicate, as was pointed out in discussing the experiment more fully described in this paper, that the mainly nitrogenous fertilizers enabled the plants to make a more normal growth than, on the whole, do the other fertilizers, although with the quantities of salts and harmful substance used in these experiments, the plants were by no means able to overcome the harmful effect entirely, if indeed this is possible.

This general relationship is even more strongly shown when the decreases in the concentrations of the solutions in these various groups are considered. The average decrease, in solutions with and without dihydroxystearic acid, for each group in each experiment, is shown in table II.

TABLE II

SHOWING THE AVERAGE DECREASES IN THE CONCENTRATION OF TOTAL CONSTITUENTS IN THE GROUP OF CULTURE SOLUTIONS CONTAINING FERTILIZER SALTS HAVING THE COMPOSITION OF 50 TO 100 PER CENT OF ANY ONE OF THE COMPONENTS P_2O_5, NH_3, AND K_2O, WITHOUT AND WITH DIHYDROXYSTEARIC ACID (ORIGINAL CONCENTRATION = 80 PARTS PER MILLION).

	50 100 PER CENT P_2O_5			50–100 PER CENT NH_3			50–100 PER CENT K_2O		
EXPERIMENT	Without	With	Decrease in dihydroxystearic acid solutions. Normal equals 100	Without	With	Decrease in dihydroxystearic acid solutions. Normal equals 100	Without	With	Decrease in dihydroxystearic acid solutions. Normal equals 100
1.....	27.8	17.6	63	35.4	24.0	68	34.0	13.9	41
2.....	28.9	24.6	55	30.3	34.6	114	38.5	30.8	80
3.....	27.8	23.8	85	35.9	32.0	89	45.0	31.0	69

The third column under each fertilizer group gives the relation between these decreases, the decrease in concentration when dihydroxystearic acid is absent being considered as the normal for comparison and equal to 100. A comparison of these relative effects shows that the mainly nitrogenous fertilizers give the highest results. In other words, the decrease with the nitrogenous fertilizers was more nearly like that observed under the normal conditions where dihydroxystearic acid was absent. This result may be interpreted to mean that the mainly nitrogenous fertilizers decreased the inhibitive effect of the dihydroxystearic acid, although it does not show whether this is a direct or an indirect effect; that is, whether there is an actual decrease of this inhibitive material or whether there is mainly the ability of the plant to withstand the attacks under these conditions. Attention has already been called to the fact that this substance when in soils is most easily destroyed by processes which promote oxidation, and it should be borne in mind that the mainly nitrogenous fertilizers are the ones which promote the most active root oxidation by the plants themselves. A study of the oxidation of roots in these

experiments showed that the dihydroxystearic acid interfered greatly with oxidation, and that this action was overcome to some slight extent in the center of the triangle but nearer to the nitrate end, thus showing perhaps that there is some correlation in these functions.

SUMMARY

The foregoing investigations have given the following results:

1. An organic soil constituent, dihydroxystearic acid, hinders the growth of wheat plants, when this is present in solution in pure distilled water.

2. The compound is also harmful in the presence of nutrient or fertilizer salts in all ratios of the fertilizer elements, P_2O_5, NH_3, and K_2O.

3. The compound is more harmful in those ratios of fertilizer elements not well suited for plant growth.

4. The harmful effect of the compound is the least in those ratios of fertilizer elements best suited for plant growth.

5. The compound appears to be relatively much less harmful in the presence of fertilizers mainly nitrogenous than in the presence of fertilizers mainly phosphatic or potassic.

6. The harmful compound modified greatly the removal of fertilizer elements from the solutions. The quantity of phosphate and potash removed was less in the presence of the compound, but the nitrate was not so influenced and on the whole the amount removed was even greater.

7. The compound modified both amount and ratio of the three fertilizer elements removed from solutions, the ratio being higher in nitrogen, which was also the most efficient fertilizer element in decreasing the harmful effect, as above mentioned.

8. The harmful compound has the additional effect of darkening the root tips, stunting root development, causing enlarged root ends, which are often turned upward like fish-hooks, and inhibiting strongly the oxidizing power of the roots.

9. Those fertilizer combinations which tend to increase root oxidation are also the combinations which overcome the harmful effects to the greatest extent.

BUREAU OF SOILS
U.S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

SOME OBSERVATIONS ON CATALASE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 139

CHAS. O. APPLEMAN

(WITH ONE FIGURE)

During the course of an investigation now in progress on the physiological behavior of enzymes in after-ripening of the potato tuber, it became necessary to investigate fully the best method for the quantitative determination of catalase in this organ. Some of the results thus obtained may be of general interest.

DISTRIBUTION AND FUNCTION OF CATALASE

SCHOENBEIN (1863) was the first to observe the power of various vegetable and animal extracts to decompose hydrogen peroxid with evolution of oxygen. He concluded that the enzymes occurring in the organisms were responsible for this phenomenon. This power of hydrogen peroxid decomposition was considered a more or less general property of enzymes until LOEW (1) showed that this property of tissues is due to a special enzyme to which he gave the name catalase. Its claim for a place in the category of enzymes at this time seems to be based wholly upon its sensitiveness toward heat, acids, and various poisons.

Catalase is probably the most widely distributed of any of the known enzymes. In fact, its occurrence is so general that LOEW concluded that there did not exist a group of organisms, or any organ, or even a single vegetable or animal cell, that did not contain some catalase. An enzyme of such general occurrence might naturally be supposed to possess an important function in the economy of nature. This may yet prove to be the case, but at present its position in this respect is uncertain. Little is even known regarding its mode of action on hydrogen peroxid. Definite knowledge on this point is limited to the fact that molecular instead of atomic oxygen results from the decomposition, and in this respect it differs from the other hydrogen peroxid catalysts.

Since the decomposition of hydrogen peroxid is the most important property of this powerful enzyme so widely distributed, it was suggested by LOEW that it may possess the function of preventing the accumulation of this toxic substance in the tissues. He conceived it possible and highly probable that hydrogen peroxid was produced in the living cells as the result of respiratory processes. USHER and PRIESTLY (9) were able to demonstrate the presence of hydrogen peroxid during photosynthesis if the catalase were previously destroyed. This fact would seem to support the above theory, but on the other hand, BACH and CHODAT (2) have shown that hydrogen peroxid is not a violent poison in tissues, since they have been able to cultivate certain plants in a medium containing 0.68 per cent of it. Catalase is found also in anaerobic organisms, a further fact which rendered the above conception untenable. Other authors have ascribed to catalase the function of protection against the peroxids of the organism, thus preventing injurious oxidations. It is unable, however, to decompose the substituted organic peroxids, such as ethyl hydroperoxid or the oxygenases (2).

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EFFECT OF GRINDING WITH CALCIUM CARBONATE

GRÜSS (**10**) reports that it is impossible to make quantitative determinations of catalase in the fresh potato extract on account of the rapid degeneration during the grinding and subsequently.

If the potato is ground with calcium carbonate to neutralize the acids freed by the grinding, and 5 cc. of the extract is at once diluted with 50 cc. and kept at a temperature of 20° C. or below, this rapid degeneration may be overcome and comparable results obtained

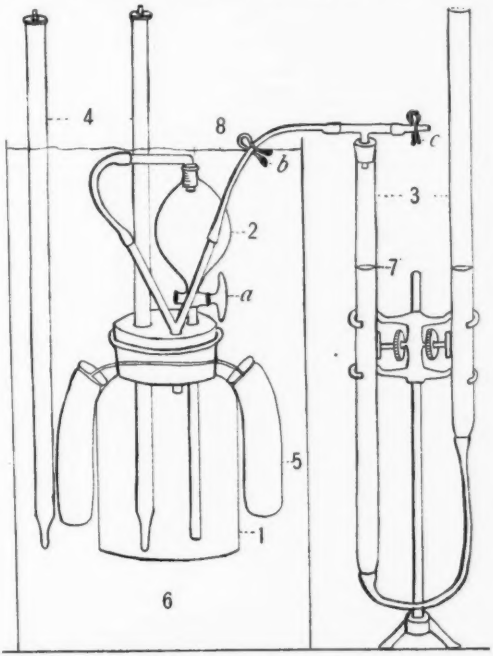

FIG. 1.—Apparatus used for catalase determinations: *1*, thin-walled bottle; *2*, separatory funnel; *3*, gas burettes; *4*, thermometers; *5*, weights; *6*, water bath; *7*, water meniscus; *8*, water level in bath; *a*, stopcock for running the hydrogen peroxid into bottle; *b*, stopcock for controlling the flow of oxygen into the burette; *c*, stopcock for equalizing the air pressure in burettes.

without any difficulty. It is necessary, however, to maintain the same period of time for grinding, as there is a slight degeneration even under the above conditions, due to another cause, as we shall see later. The shaking must also be constant and uniform during the determination.

Table II shows the effect of grinding and storage with calcium carbonate. After grinding two potatoes of equal weight, one with

that there is a tendency for the area of greatest growth under these conditions to be nearer this end.

In other words, when dihydroxystearic acid is present the results show a tendency for the ratios of the materials removed from the solution to fall nearer to the nitrogen end of the diagram than they do in the case of cultures where this substance is absent. This

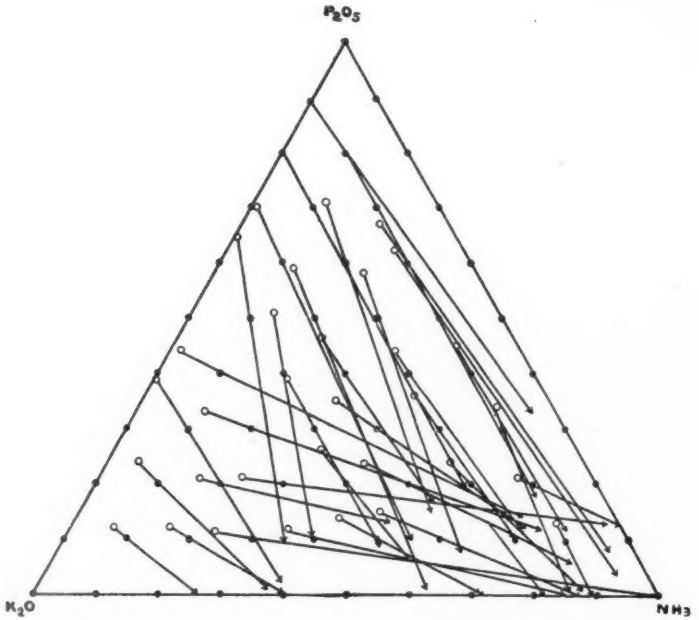

FIG. 9.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the culture solution, in the first period.

tendency is marked from the very first, as is shown by the diagram given in fig. 9 for the first period. Moreover, the points, for instance, of the 10 per cent mixture of P_2O_5 lie very low, and this tendency to lie lower than in the cultures which contained no dihydroxystearic acid is found throughout the experiment, though it is not equally marked in all periods. The average effect has already been given in the diagram fig. 8. The strong tendency

which is shown when this substance is present for a proportionately, and sometimes even absolutely, greater decrease in the nitrogen of the solutions, is strikingly shown in this first period (fig. 9). This varies somewhat from period to period; thus, for instance, in the fourth period, shown in fig. 10, the ratio change is fairly normal, though the usual tendency is seen again in the seventh period, a

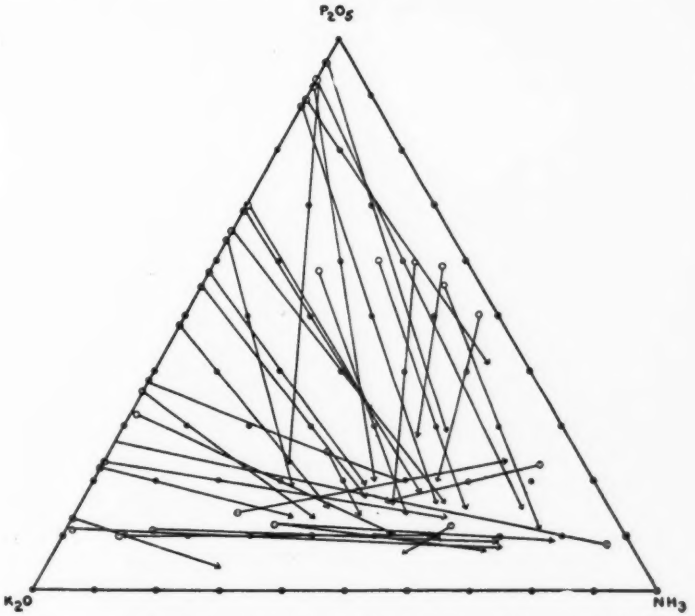

FIG. 10.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the solution, in the fourth period.

diagram of which is presented in fig. 11. These diagrams for the several periods illustrate rather well the general tendencies brought out by an examination of the analytical data. As a rule, beyond the second or perhaps the third period the diagrammatic representation of the result is on the whole uniform, but is influenced undoubtedly by the conditions of growth during any period; in other words, by weather and other conditions, which is shown perhaps quickest

in the nitrate removal from the cultures. The influence of light conditions on different days has already been discussed in the previous paper.

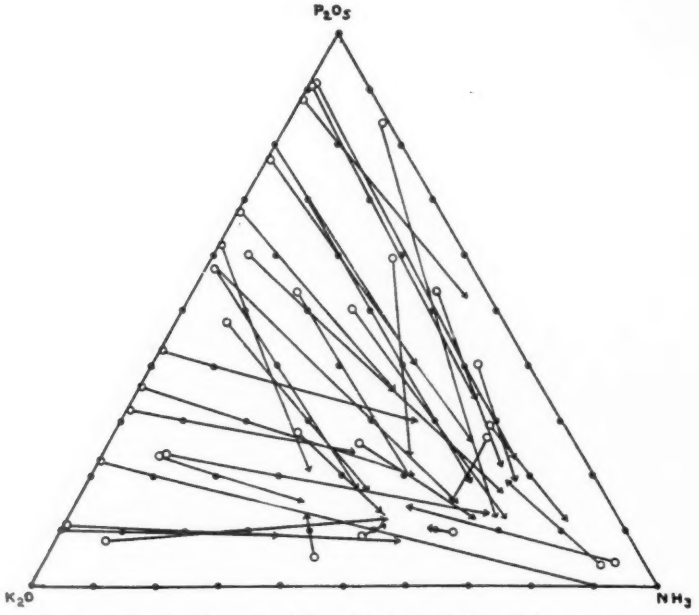

FIG. 11.—Showing the ratio of the original, the final, and the ratio of the loss of P_2O_5, NH_3, and K_2O from the solution, in the seventh period.

THE COMPARATIVE EFFECT PRODUCED ON THE DIHYDROXYSTEARIC ACID CULTURE SOLUTIONS BY THE FERTILIZER MIXTURES COMPOSED MAINLY OF P_2O_5, NH_3, AND K_2O RESPECTIVELY

In the experiment presented, as well as in two others in which both sets of cultures were grown simultaneously, there was noticed a more general tendency toward normal development of the plants in the presence of dihydroxystearic acid when the culture solutions contained all three of the fertilizer substances. It was further apparent that there seemed to be a tendency for more normal development in a region nearer to the nitrate end. In order to

trace out this matter, a comparison of the three experiments has been made and is hereby presented. In this way the solutions which contained in all cases 50 per cent or more P_2O_5 are considered in one group; all those which contained 50 per cent or more NH_3 in a second group; and those with 50 per cent or more K_2O in a third group. By grouping the cultures thus, an average result is obtained with the mainly phosphatic, mainly nitrogenous, and mainly potassic fertilizers.

A comparison of the average green weight in each of these groups of cultures was made with those in the corresponding groups in the cultures where dihydroxystearic acid was absent. The relative growths thus obtained, taking the growth without the dihydroxystearic acid as 100, are contained in table I.

TABLE I

SHOWING THE AVERAGE RELATIVE GROWTH MADE IN THE GROUP OF SOLUTIONS WITH DIHYDROXYSTEARIC ACID AND CONTAINING FERTILIZER SALTS HAVING THE COMPOSITION OF 50 TO 100 PER CENT OF ANY ONE OF THE COMPONENTS P_2O_5, NH_3, AND K_2O.

EXPERIMENT	RELATIVE GREEN WEIGHT (Green weight without dihydroxystearic acid = 100)		
	P_2O_5 50–100 per cent	NH_3 50–100 per cent	K_2O 50–100 per cent
1	49	67	43
2	60	62	62
3	71	68	61

In the first column is given the number of the experiment; in the second column the average relative green weight obtained in the solutions having mainly phosphatic fertilizers; in the third column the same result for the cultures of mainly nitrogenous fertilizers; and in the fourth column the results obtained when mainly potassic fertilizers were present. The results indicate, as was pointed out in discussing the experiment more fully described in this paper, that the mainly nitrogenous fertilizers enabled the plants to make a more normal growth than, on the whole, do the other fertilizers, although with the quantities of salts and harmful substance used in these experiments, the plants were by no means able to overcome the harmful effect entirely, if indeed this is possible.

This general relationship is even more strongly shown when the decreases in the concentrations of the solutions in these various groups are considered. The average decrease, in solutions with and without dihydroxystearic acid, for each group in each experiment, is shown in table II.

TABLE II

SHOWING THE AVERAGE DECREASES IN THE CONCENTRATION OF TOTAL CONSTITUENTS IN THE GROUP OF CULTURE SOLUTIONS CONTAINING FERTILIZER SALTS HAVING THE COMPOSITION OF 50 TO 100 PER CENT OF ANY ONE OF THE COMPONENTS P_2O_5, NH_3, AND K_2O, WITHOUT AND WITH DIHYDROXYSTEARIC ACID (ORIGINAL CONCENTRATION = 80 PARTS PER MILLION).

	50 100 PER CENT P_2O_5			50–100 PER CENT NH_3			50–100 PER CENT K_2O		
EXPERIMENT	Without	With	Decrease in dihydroxystearic acid solutions. Normal equals 100	Without	With	Decrease in dihydroxystearic acid solutions. Normal equals 100	Without	With	Decrease in dihydroxystearic acid solutions. Normal equals 100
1.....	27.8	17.6	63	35.4	24.0	68	34.0	13.9	41
2.....	28.9	24.6	55	30.3	34.6	114	38.5	30.8	80
3.....	27.8	23.8	85	35.9	32.0	89	45.0	31.0	69

The third column under each fertilizer group gives the relation between these decreases, the decrease in concentration when dihydroxystearic acid is absent being considered as the normal for comparison and equal to 100. A comparison of these relative effects shows that the mainly nitrogenous fertilizers give the highest results. In other words, the decrease with the nitrogenous fertilizers was more nearly like that observed under the normal conditions where dihydroxystearic acid was absent. This result may be interpreted to mean that the mainly nitrogenous fertilizers decreased the inhibitive effect of the dihydroxystearic acid, although it does not show whether this is a direct or an indirect effect; that is, whether there is an actual decrease of this inhibitive material or whether there is mainly the ability of the plant to withstand the attacks under these conditions. Attention has already been called to the fact that this substance when in soils is most easily destroyed by processes which promote oxidation, and it should be borne in mind that the mainly nitrogenous fertilizers are the ones which promote the most active root oxidation by the plants themselves. A study of the oxidation of roots in these

experiments showed that the dihydroxystearic acid interfered greatly with oxidation, and that this action was overcome to some slight extent in the center of the triangle but nearer to the nitrate end, thus showing perhaps that there is some correlation in these functions.

SUMMARY

The foregoing investigations have given the following results:

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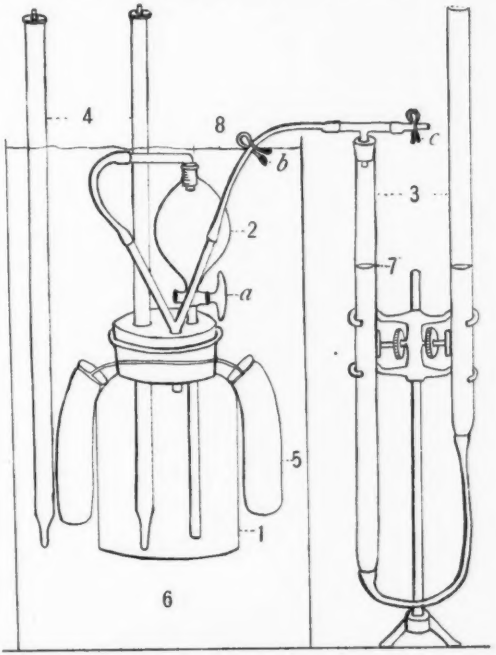

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Table II shows the effect of grinding and storage with calcium carbonate. After grinding two potatoes of equal weight, one with

and the other without calcium carbonate, they were stored at 0° C. for 34 days. At the end of this time, 200 cc. of water were

TABLE I

LOSS OF CATALASE DUE TO ACIDS IN THE EXTRACT

POTATO	CC. O_2 EVOLVED IN 1-MIN. PERIODS					TOTAL CC. O_2 IN 5 MIN.
	1st	2d	3d	4th	5th	
Ground without $CaCO_3$....	3.2	3.0	2.3	1.7	1.3	11.5
Same extract after standing 15 min.....	1.7	1.8	1.6	1.3	1.0	7.4
Ground with $CaCO_3$.....	6.4	5.9	4.6	3.6	2.8	23.3
Same extract after standing 90 min.....	6.5	5.8	4.3	3.3	2.6	22.5

added to each flask containing the pulp, and 5 cc. of this dilution and 5 cc. of hydrogen peroxid were used for the determination in each case.

TABLE II

EFFECT OF STORAGE FOR 34 DAYS AT 0° C. WITH AND WITHOUT CALCIUM CARBONATE

POTATO	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
Ground without $CaCO_3$.....	0.3	0.4	0.4	0.4	0.4	0.4	2.3
Ground with $CaCO_3$	12.4	10.9	8.9	6.9	5.4	4.0	48.4

FILTERING

Approximately 50 per cent of the total catalase will pass through an ordinary filter paper, but none through a Chamberland-Pasteur filter. The first fact seems to indicate the existence of an insol-

TABLE III

EFFECT OF FILTERING THROUGH ORDINARY FILTER PAPER

EXTRACT OF POTATO TUBER	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
Filtered through filter paper	2.8	2.4	1.8	1.5	1.3	1.1	10.9
Portion of same extract unfiltered.....	4.0	4.5	3.8	3.2	3.1	3.4	22.6

uble (α-catalase) and a soluble (β-catalase) form. KOHL finds the same true of yeast catalase, and LOEW believes it a general char-

acter of vegetable catalase. The size of the molecule of the latter prevents its passage through a Chamberland-Pasteur filter.

Table IV also shows the effect of filtering and the necessity of thorough mixing of the extract before taking the sample for a total catalase determination.

TABLE IV

EFFECT OF FILTERING AND SETTLING

EXTRACT OF POTATO TUBER	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
Unfiltered	12.2	9.5	7.0	5.0	4.0	3.2	40.9
Supernatant liquid after settling	5.4	4.5	3.5	3.0	...	...	....
After mixing, 20 min. later	11.9	9.5	6.9	5.3	3.8	3.2	40.6
Filtered, 40 min. later	5.5	4.8	3.5	2.7	2.1	1.7	10.3

TABLE V

EFFECT OF FILTERING THROUGH A CHAMBERLAND-PASTEUR FILTER

EXTRACT OF POTATO TUBER	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st	2d	3d	4th	5th	6th	
a) Unfiltered	24.8	22.8	19.3	16.8	14.8	13.5	52.
b) Filtered through Chamberland filter	0.	0.	0.	0.	....	....	....
c) Residue of (*b*) made up to same volume as (*a*)	25.	22.3	19.3	16.7	14.8	13.5	51.6

TEMPERATURE RELATIONS

Catalases from different sources show considerable variation in temperature relations, the point of total destruction in the cases reported ranging from 65° C. to 80° C. In potato catalase, however, destruction is complete when the temperature reaches 50° C.

The VAN'T HOFF velocity coefficient for hemase has been found to be 1.5. The same figure applies to potato catalase, but is evident only from 0 to 10°. At 20° a destruction of the catalase begins, which renders the accelerating effect of higher temperatures upon the peroxid decomposition impossible of manifestation.

The diminishing coefficient indicated in table VI is due to actual destruction of the catalase, as will be seen by table VII, which also shows that the destruction at moderate temperatures is not due

to impurities in the hydrogen peroxid. Some substance in the potato may be freed by the grinding and brought into contact

TABLE VI

JOINT EFFECT OF ACCELERATION AND DESTRUCTION BY RISING TEMPERATURE

Range of temperature	0–10°	10–20°	20–30°	30–40°
Velocity coefficients	1.5	1.1	0.85	0.18

with the catalase, which effects its slow destruction. It is interesting to note in this connection, however, that SENTER (**6**) found hemase to be oxidized at all temperatures above 0° C.

TABLE VII

AMOUNT OF CATALASE DESTRUCTION AT 30° C. FOR 15 MIN. OVER THAT AT 20° C. FOR THE SAME PERIOD

POTATO TUBER EXTRACT	DETERMINATION TEMP.	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
		1st	2d	3d ½	4th	5th	6th	
a) Exposed to 30° C. only during determination of catalase	30° C.	14.8	14.7	14.1	13.2	12.5	11.9	21.2
b) Exposed to 30° C. during exp. (*a*), then cooled to 20° C. for determination	20° C.	15.0	14.5	13.7	13.3	12.7	12.4	21.6
c) Not exposed above 20° C	20° C.	15.9	15.1	14.7	13.9	13.3	12.8	25.7

DESTRUCTION OF THE CATALASE DURING THE REACTION WITH HYDROGEN PEROXID

Potato catalase is not unlimited in its power to effect the decomposition of hydrogen peroxid. Table VIII shows not only the above fact, but also would seem to indicate that it is consumed in the reaction and that a given amount is capable of decomposing a definite amount of hydrogen peroxid.

The total extract of a potato weighing 50 grams was diluted to 500 cc. with water, and 5 cc. of this extract were allowed to act upon 1 cc. of 1.5 per cent hydrogen peroxid until less than 0.1 cc. of oxygen was evolved in 5 min. Another cubic centimeter of hydrogen peroxid was then added and the reaction allowed to run to the same point. This procedure was continued until the addition of a cubic centimeter of hydrogen peroxid produced only 1.1 cc. of oxygen in 25 min.; 6 cc. were required to bring the reaction to

this point. A fresh portion of 5 cc. of the extract, which had stood at the same temperature, was now allowed to act upon 6 cc. of hydrogen peroxid at the same time, with the result that approximately the same amount of oxygen was evolved as the total produced when 6 cc. were added in successive lots of 1 cc. each. The experiment was repeated several times with different extracts and dilutions, with practically the same result in every case.

TABLE VIII

CONSUMPTION OF THE CATALASE DURING THE REACTION WITH HYDROGEN PEROXID

POTATO TUBER EXTRACT	CC. O_2 EVOLVED WHEN H_2O_2 WAS ADDED IN SUCCESSIVE LOTS OF 1 CC. EACH						TOTAL CC. O_2 EVOLVED	TOTAL CC. O_2 EVOLVED WHEN 6 CC. WERE ADDED IN BULK
	1st	2d	3d	4th	5th	6th		
5 cc. extract	8.2	6.5	6.3	5.4	4.0	1.1	31.6	
5 cc. same extract	...	...	...	...	...	...	....	31.8

The destruction or degeneration of the catalase would be slight during the time of the experiment in the above dilute neutral solution at 20°. Admitting a slight destruction, it would be the same in both cases, and would therefore not affect the general result. KASTLE (**7**) suggests that catalases, like peroxidases, may combine with the hydrogen peroxid to form an unstable holoxid derivative, as $K+H_2O_2=H_2KO_2$. This might in turn oxidize the catalase itself (K) which may be more readily oxidizable than any of the peroxidase reagents, in which event we would have $H_2KO_2=H_2O+KO$. This conception is in striking harmony with the facts indicated in the above table, and with the fact that the reaction is decidedly an exothermal one.

RELATION TO FUNCTIONAL ACTIVITY

There is considerable evidence from animal tissues that catalase activity bears a relation to functional activity of the structure. Such a relation seems to exist in the potato, at least in respect to respiratory activity. Extracts from potatoes which have been kept for several days at 0° C. show a decided decrease in catalase activity. MÜLLER-THURGAU (**8**) has also shown that respiration is greatly reduced in such potatoes.

Catalase activity is greater at the end than at the beginning of the rest period. It is also greater in the large mature potatoes

TABLE IX

DECREASE IN CATALASE ACTIVITY AFTER STORAGE AT 0° C.

WEIGHT OF WHOLE POTATO	TIME OF STORAGE	TEMP. OF STORAGE	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
			1st	2d	3d	4th	5th	6th	
219 gm.....	28 days	20° C.	12.8	10.5	7.5	5.5	4.3	3.8	44.4
214 gm.....	28 days	0° C.	7.5	6.9	5.2	4.0	3.1	2.6	29.3

than in the small immature ones. This is true to a much greater extent in new potatoes than old ones. MÜLLER-THURGAU (**8**) found that these same relations exist in regard to respiration.

TABLE X

RELATION OF CATALASE ACTIVITY TO AGE AND SIZE OF THE POTATOES

POTATO AT	WT. OF POTATO	CC. O_2 EVOLVED IN 30-SEC. PERIODS				TOTAL CC. O_2 IN 2 MIN.
		1st	2d	3d	4th	
Beginning of rest period ..	100 gm.	17.5	16.3	14.5	13.6	21.9
Beginning of rest period ..	400 gm.	27.3	23.8	19.9	16.9	47.9
End of rest period.....	147 gm.	35.5	26.0	19.1	15.0	55.6
End of rest period.....	540 gm.	39.0	27.5	19.1	14.5	60.1

In table XI equal weights of morphologically similar pieces of new and old potatoes of about the same weight were ground, washed into a volumetric flask, and made up to 250 cc. with water. After thorough shaking, 5 cc. were withdrawn for the test, using 5 cc. of hydrogen peroxid as before.

TABLE XI

CATALASE ACTIVITY AT BEGINNING AND END OF REST PERIOD

POTATO AT	CC. O_2 EVOLVED IN 30-SEC. PERIODS						TOTAL CC. O_2 IN 3 MIN.
	1st]	2d	3d	4th	5th	6th	
Beginning of rest period	16.5	16.0	14.8	14.0	13.5	12.9	27.7
End of rest period.....	21.2	20.2	18.0	16.1	14.7	13.8	44

The above facts do not prove a causal relation between catalase and respiration, but they are highly suggestive, especially in the

light of KOHL's recent claims for catalase as an important enzyme in alcoholic fermentation, and therefore most likely in respiratory processes.

SUMMARY

1. Comparable quantitative determinations of potato catalase can be made in the fresh extracts, if the material is ground with calcium carbonate, the extract diluted immediately with ten parts of water to one of extract, kept at 20° C. or below, and the same time maintained for the grinding of the potato and the catalase determination.

2. An insoluble (α-catalase) and a soluble (β-catalase) form may be separated by ordinary filter paper. Approximately 50 per cent of the total passes through. None will pass through a Chamberland-Pasteur filter.

3. The VAN'T HOFF velocity coefficient for potato catalase is 1.5 from 0° C. to 10° C. At higher ranges of temperature there is an apparent progressive decrease in the velocity coefficient. This is due to actual destruction of the catalase, which is not due in the main to impurities in the hydrogen peroxid or to oxidation by the hydrogen peroxid.

4. Potato catalase is not unlimited in its power to effect the decomposition of hydrogen peroxid. It seems to be consumed in the reaction and a given amount is capable of decomposing a definite amount of hydrogen peroxid.

5. The catalase activity bears a relation to the respiratory activities in the potato, decreasing under the same conditions as respiration.

I wish to acknowledge my indebtedness to Dr. WILLIAM CROCKER, under whose supervision this work was pursued.

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TWIN HYBRIDS (*LAETA* AND *VELUTINA*) AND THEIR ANATOMICAL DISTINCTIONS

FRANK M. ANDREWS

Twin hybrids are produced according to DEVRIES "by the combinations *Oenothera biennis*×*O. Lamarckiana*, and *O. muricata*×*O. Lamarckiana*, and by those of some of their derivatives."[1] Besides *laeta* and *velutina* I shall describe here *O. Lamarckiana* and *O. biennis*. *O. biennis*, along with some other species, was introduced into Europe a long time ago. The origin of *O. Lamarckiana*, or large-flowered evening primrose, is involved in obscurity. In an old potato field near Hilversum, in the year 1886, DEVRIES[2] found a great many specimens of *O. Lamarckiana* which were observed to be in a state of mutability. I have visited this primrose field to observe and collect specimens. There are comparatively few of them left, due to the fact that they are rapidly being crowded out by pine trees that have been planted there. It is apparently only a question of a little time, unless something intervenes, until they will have disappeared entirely from that locality. *O. Lamarckiana* produced new forms by mutation, not only in the wild state in the field near Hilversum, but also in cultivation. This latter fact was proved by transferring rosettes from the field to the garden, where the plants could be protected and watched throughout their entire existence. This method, together with that of sowing the seeds[3] of these plants under controlled conditions, has allowed quite a number of mutants to assert themselves and to be recognized. Unless such methods as were introduced by DEVRIES in his garden at Amsterdam are followed, the detection of a mutation is much more apt to be only accidental. Furthermore, these methods when used for rapid multiplication in the garden under controlled and protected conditions may also be conducive to an increase of mutants, and hence their more certain and frequent recognition. For "such a rapid multiplication in the course of a comparatively few years" (as

[1] DEVRIES, HUGO, On twin hybrids. BOT. GAZETTE **44**:401–407. 1907.

[2] DEVRIES, HUGO, Mutationstheorie **1**:152, 401–407.

[3] DEVRIES, HUGO, Species and varieties, p. 525. 1905.

was noticed with *O. Lamarckiana* near Hilversum) "may perhaps be," says DEVRIES, "one of the conditions for the appearance of a mutable period."[4] The number of new forms that have arisen and been detected by DEVRIES in the genus *Oenothera* since his discovery of the mutating *O. Lamarckiana* at Hilversum in 1886 is already considerable.

The following description of the forms *O. Lamarckiana*, *O. biennis*, *laeta*, and *velutina* represents in each case the average state of affairs as shown by a great number of examined specimens.

O. Lamarckiana is an erect, strong plant. Its stem is cylindrical, 1–1.5 cm. in diameter, 1.5 or more meters in height, and sparsely covered with coarse hairs. Some of the hairs on the stems are glandular. The main stem is more or less branched at the top, and also has a tendency to produce smaller branches at the bottom when it grows in an open situation. All of the secondary branches may produce flowers.

The leaves are somewhat broadly lanceolate, dark green, their margins distinctly dentate, and the lower ones strongly petioled. Both the upper and the lower surfaces, especially in the adult forms, are conspicuously undulated or wrinkled, and only slightly pubescent.

In order to get buds showing as great uniformity as possible, they were chosen just before they were ready to open. The buds of *O. Lamarckiana* are the largest of any of the three forms mentioned, being about 5.5 cm. in length, about 1 cm. broad near the base, and tapering slowly upward to a width of about 2 mm. at the top. They are slightly pubescent. This pubescence of the buds of all the three forms studied consists of two kinds of hairs, a small, thin-walled, cylindrical hair, and a larger, thick-walled, pointed one. In *O. Lamarckiana* both of these hairs are much larger than in any of the other forms of *Oenothera* to be described, and especially is this true of the pointed hairs. The buds are almost circular in cross-section.

The flowers are large and bright yellow, and arranged rather closely together in a broad spike. Each flower is subtended by a sessile bract and stands singly. The ovary is partly glandular-pubescent, and the four lanceolate sepals are strongly reflexed, gen-

[4] DEVRIES, HUGO, Mutationstheorie 1:152.

erally more or less united at the top, and somewhat pubescent. The calyx tube is a little longer than the sepals. The cell walls of the epidermis of the sepals of *O. Lamarckiana*, especially the inner epidermis, are very irregular and zigzag in form. At the angles of these zigzag places the walls of the inner epidermis are marked by conspicuous thickenings. In the case of the outer epidermis the thickenings at these places extend away from the main wall as T-shaped processes. The same is true, but to a slightly less degree, for the inner and outer epidermis of the petals. The four very large, broadly obovate, entire petals are very conspicuous, and almost or quite equal the length of the calyx tube. One of the distinguishing features of *O. Lamarckiana* is the tall stigma, which far exceeds in height the stamens, and on this account self-pollination cannot occur. The anthers of *O. Lamarckiana* open in damp or rainy weather as well as on clear days, but this, as we shall see later, is not true of some forms, as for example the twin hybrids. This can be accomplished in the parent species, *O. Lamarckiana*, because at maturity the tissues of the cell walls of the anther are completely differentiated and become free, so that the outer wall may open out in the usual way.

The capsules are four-celled and open by as many valves at maturity. The pubescence on the capsules is not so dense as on the ovary. As the old flowers disappear and new ones appear, long rows of capsules are left on the older parts of the spike.

Oenothera biennis is not as stout as *O. Lamarckiana*. Its stem is erect, cylindrical, branching, and sparsely covered with coarse but non-glandular hairs. It is branched, is about 1 cm. in diameter, and 1–1.5 meters in height.

The leaves are dark green, broad (somewhat more so than in *O. Lamarckiana*), about three times as long as wide, with margins slightly wavy, and all of the stem leaves are petioled. In the last two respects the Holland species is somewhat different from the more narrow-leaved American species of *O. biennis*, which has only the "lowest petioled"[5] and the margins "repand-denticulate."[5] These are among the differences that show that the form *O. biennis*, which is very common in the United States, is not the same as the *O. biennis*

[5] BRITTON AND BROWN, Flora of the northern U.S. and Canada **2**:486.

of Europe. The upper and lower surfaces of the lower leaves are not wrinkled as we have seen in *O. Lamarckiana*, and are less hairy.

The buds are much smaller than those of *O. Lamarckiana*, averaging 1.5 cm. long, 6 mm. wide at the base, and 1.5–2 mm. wide at the top. Besides being smaller, they are different in form from those of *O. Lamarckiana*. One side of the bud is rather straight, and the other more or less gibbous. Comparing this with the American species, as shown by BRITTON and BROWN'S figure,[6] there is another difference, for in the American forms the buds are larger and more pointed, especially at the apex, than in the Holland type. The hairs on the buds are only about half the size of those of *O. Lamarckiana*.

The flowers are much smaller than those of *O. Lamarckiana* and are bright yellow. Each flower stands in the angle of a nearly sessile bract on the rather narrow spike. The calyx tube in this form is 1.5–2 times the length of the reflexed sepals. The density of the pubescence on the sepals is about the same as for *O. Lamarckiana*. The irregularity shown in the epidermal cell walls of the sepals is much less in *O. biennis* than in *O. Lamarckiana*, and especially is this true of the inner epidermal cell walls, which are only wavy, and in which the thickenings are almost completely absent. The cells of the outer epidermis are also decidedly smaller and have but few thickened places.

The four petals are broad, slightly unequally lobed, somewhat indented at the apex, and larger than the sepals. The cells both of the inner and outer epidermis are smaller, more regular in form, and are, especially those of the inner epidermis, almost devoid of the angular thickenings shown by *O. Lamarckiana*.

One of the chief differences in the flowers of *O. Lamarckiana* and *O. biennis* is that in the latter the stamens are of the same height as the stigma, and the anthers are in contact with the stigma in the bud. Therefore self-pollination nearly always results, whereas in *O. Lamarckiana* it was generally brought about by insects. The four anther cells do not always open simultaneously, but some may have partly opened and shed their pollen before the opening process in the other cells has begun. The ovary is four-celled and pubescent.

[6] BRITTON AND BROWN, *op. cit.* 2:486. *fig.* 2579.

The capsules are almost pressed against the stem. They are about 24 mm. long, somewhat pubescent, and remain as the flowers disappear in long rows on the spike, as in *O. Lamarckiana*. These capsules of *O. biennis*, according to BRITTON and BROWN's figure,[7] seem to be differently curved and more pointed toward the top. Instead of the usual number of parts of the flower of *O. biennis*, I have observed some where there were three sepals, three petals, six stamens, and three stigmas. Deviations of a similar kind I have noticed in other plants.[8]

The stem of *laeta* is branched, about the same height as that of *O. biennis*, and almost intermediate between the two parents as to stoutness and pubescence.

The leaves are broad, bright green, and while wrinkled they are hardly as much so as in *O. Lamarckiana*. They are considerably more pubescent than those of *O. biennis* and slightly more so than those of *O. Lamarckiana*.

The buds differ in size and form from those of the other forms. They are more pubescent than in *O. Lamarckiana* or *O. biennis*, smaller than in *O. Lamarckiana*, and larger than in *O. biennis*. In cross-section they are almost round.

The hairs of the buds and other parts of the plant are distinctive as to size, for they are considerably longer than those of either *O. biennis* or *velutina*, but smaller than those of *O. Lamarckiana*.

The lanceolate and strongly reflexed sepals are about three-fourths of the length of the calyx tube, and are somewhat more pubescent than in any of the preceding forms.

Both the form and the size of the cells of the outer and inner epidermis of the sepals, as well as of the petals, of *laeta* are different from those of either of the three other forms mentioned here.

The relative size of the stamens and style is another distinctive feature, for the style is somewhat higher than the stamens, being less pronounced than in *O. Lamarckiana*. Both the stamens and the style are much smaller than in *O. Lamarckiana*. The stamens about equal in size those of *O. biennis* and *velutina*. The anther cells behave differently from any of the previously described forms, in

[7] BRITTON AND BROWN, *op. cit.* 2:486. *fig.* 2579.

[8] See my paper in Proc. Indiana Acad. Sci. for 1905 and 1906.

that in damp or rainy weather they do not open at all, and sometimes very imperfectly even on sunny days. I have observed anthers, one or two of whose cells never open, and the remaining cells do so only partly. The anthers seem to develop normally in every respect except that the tissues do not become differentiated completely at the usual point of opening, so as to allow the escape of the abundantly formed pollen grains in the ordinary way. It was often necessary to exert strong pressure on the cover glass before the anther wall would separate at the usual place, and this was true even in thin cross-sections.

The ovary of *laeta* is more pubescent than that of *O. Lamarckiana* or *O. biennis*.

The capsule is about the size of that of *O. Lamarckiana*, but different in form from either parent. They form very long spikes of fruit before the close of the flowering period.

Velutina, the other twin hybrid form, is also distinctly different from *laeta* and all other forms.

Its stem is about the same height as that of *laeta*, somewhat more branched, and light grayish, due to the dense pubescence. The hairiness of the whole plant is much more pronounced than in any of the other forms described, and this fact alone would serve to distinguish it from any of them. The stem is not quite so stout as that of *laeta*, and the aspect of the whole plant is somewhat less vigorous.

The leaves are grayish green, narrow (more so than in *laeta*), trough-shaped, tapering toward each end, and much more pubescent than the leaves of any of the other forms.

The buds are shorter than those of *laeta*, but have a greater diameter and are much more hairy. The hairs from the buds, as in the other types, are of two forms, cylindrical and pointed. The cylindrical hairs are only a little larger than those of *O. biennis*, but smaller than in the other types. The pointed hairs are exceeded in size by those of *O. Lamarckiana*, but are larger than those of *O. biennis* and *laeta*. These pointed hairs of *velutina* differ from those of *laeta* and all the other forms in another respect. In all the other forms the pointed hairs are smooth, but in *velutina* they are densely covered by numerous and conspicuous elevations.

The flowers are somewhat smaller than those of *laeta*.

The sepals are only about one-half the length of the calyx tube, whereas in *laeta* they are about three-fourths of its length. This difference is very evident on comparison. The sepals of *velutina* are also much more hairy than any of the other forms.

The cells of the outside epidermis of the sepals of *velutina* are about uniform in size with those of *laeta*, but the walls are much stronger. The cells of the inner epidermis are more uniform in size than in *laeta*.

The petals are more deeply indented at the top than in *laeta*, are somewhat smaller, and light yellow. The cells of the inner and outer epidermis are more nearly isodiametric than in *laeta*, and their cell walls are much more zigzag, and thickened at the angles.

The stamens are the same length as the stigmas. In this respect they differ strikingly from *laeta* and *O. Lamarckiana*, but resemble *O. biennis*. As in *laeta*, they open some of their anther cells at least, on sunny days, but on damp and rainy days they remain closed. I have noticed instances, as in *laeta*, where some of the anther cells never open, no matter how ideal the conditions. I have found it necessary to use considerable force in order to cause even thin cross-sections of the anthers to open their cells. This, as in *laeta*, is due to the fact that the tissues at the point of opening never separate, but remain more or less completely grown together. This non-opening of the anthers of *Oenothera* forms, however, is not confined to those mentioned above. I have visited another primrose garden at Haarlem which is conducted by Mr. A. R. SCHOUTEN and Mr. J. JESWIET. In this garden are grown, among other forms, *O. scintillans* and *O. gigas*, and Mr. JESWIET kindly informed me that they often find it necessary to open the anthers with a needle in order to free the pollen. Much of this pollen, as in our twin hybrids, is not sterile, but in some cases would never escape naturally from the anthers. Mr. JESWIET tells me that of the types they have at Haarlem all of the anthers of *O. scintillans* must be opened with a needle to obtain the pollen, and also many of the anthers of the form *O. gigas*.

The ovary of *velutina* exceeds all the other forms as to density of pubescence.

The capsule resembles that of *laeta*, but is curved near the top. It is strikingly different from *laeta* in regard to its denser pubescence.

One further difference remains to be recorded in reference to the petals of the flowers of *O. Lamarckiana*, *O. biennis*, *laeta*, and *velutina*. As we look at these flowers on their respective plants, the smaller and even the more striking differences of the petals are apt to escape notice. These can be seen most clearly by removing a flower of each of the forms just named and spreading them out carefully on white paper. To do this best and to fasten them to the paper, a coat of mucilage is first applied. This allows the petals to be arranged without injury, and at once discloses their true size, form, and relation to each other.

In *O. Lamarckiana* the four large petals do not touch one another even at their nearest edges. Their narrowed bases leave wedge-shaped openings. The lobes of the petals are equal.

In *O. biennis* the form of the petals is at once seen to be different, and they do not touch each other. The petals do not run down to the base as straight lines, but curve in so as to leave a nearly flask-shaped opening. Furthermore, the petals are slightly unequally lobed, but always in one direction.

Laeta is very plainly different from *velutina* by the conspicuous and considerable overlapping of its four petals. These overlapped edges of the petals leave inclosed spaces at their base. These petals are also seen to be considerably larger than those of *velutina*.

The petals of *velutina* resemble those of *O. biennis* in general form, but are somewhat unequally lobed and larger. The spaces between the petals of *velutina* are larger and more nearly closed by the broad part of the petal than in *O. biennis*.

The cross-sections of the stamens mentioned in this paper were made as follows: Holes were made in cork with a small cork-borer, the anthers were placed carefully in them, and the holes were then filled with liquid glycerin jelly. After the glycerin had again become firm, the piece of cork with the specimens was placed in 96 per cent alcohol and allowed to dehydrate and further solidify the glycerin, which required two to three days. The sections were then made through both cork and specimens free-hand with a razor, and mounted for study in glycerin jelly. Such specimens, of course, can be made permanent by sealing the cover glass with Canada balsam.

Summary

1. The stigma of *Oenothera Lamarckiana* is much higher than the anthers. The anthers open both on rainy and sunny days, whereas some of the other forms open their anthers only on sunny days.

2. The European species, *O. biennis*, differs from the American species. Its anthers and stigma are of the same height, instead of being of different height as in *O. Lamarckiana*. Its petals, pubescence, and most of its cells are the smallest of any of the forms described, while those of *O. Lamarckiana* tend to be the largest.

3. The twin hybrids *laeta* and *velutina* show themselves by their foliage, flowers, the greater density and character of the pubescence in *velutina*, as well as the arrangement and form of the cells, to be distinct, and, in so far as has yet been investigated, constant forms.

4. On rainy days the anthers of the twin hybrids do not open, but only on sunny days, and then only part of them, whereas in all the other forms they open on both rainy and sunny days. This non-opening of the anther cells may probably be the beginning of sterility.

In conclusion I take pleasure in expressing my thanks to Professor DeVries for having placed at my disposal one of his laboratories and material from his garden for this investigation, and also for his many kind suggestions and assistance.

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AN EOCENE FLORA IN GEORGIA AND THE INDICATED PHYSICAL CONDITIONS[1]

EDWARD W. BERRY

(WITH TWO FIGURES)

Eocene plants have been thus far unknown along the Atlantic border, the most easterly known flora of this age having been that of the so-called Eolignitic of the Mississippi embayment, which while abundant is for the most part uncollected and undescribed. Contrasted with this paucity of eocene plants in the east, the western interior Eocene has a flora which in number of species probably exceeds the sum total of all the later tertiary floras of North America. There are also a number of eocene floras along the Pacific coast and in the far north as far as Alaska, the mouth of the Mackenzie River, and Greenland.

The following brief paper is concerned with a most interesting eocene flora of Claiborne age recently discovered, and studied by the writer in connection with the cooperative investigation of the coastal plain in charge of Dr. T. WAYLAND VAUGHAN of the U.S. Geological Survey. These notes partake of the nature of an abstract of this study, which will eventually be published in full by that organization.

The localities are all in eastern Georgia, where there is a marked transgression of the Claiborne sediments, burying all traces of Upper Cretaceous or earlier Eocene deposits and coming to rest upon beds of Lower Cretaceous age or even in some instances upon the crystalline rocks of the eastern Piedmont. The bulk of the plants come from the vicinity of Grovetown, about seventeen miles west of Augusta near the southeastern border of Columbia County. In this area the deposits, which consist for the most part of porous laminated light-colored clays alternating with heavy beds of sand, and more rarely beds of lignite, occupy a pre-Claiborne estuary eroded in the Lower Cretaceous and outcropping as a narrow tongue only two or three miles in width, and extending from the

[1] Published by permission of the Director of the U.S. Geological Survey.

main body of the Claiborne deposits in Richmond County in a northwesterly direction for a distance of about eighteen miles.

The plants are intimately associated with a few estuarine and shallow water marine invertebrates, such as *Modiolus*, *Ostrea*, *Nucula*, *Leda*, *Cytherea*, etc., which fully corroborate the age of the deposits derived from the study of the flora, where the data for correlation, in the absence of known Claiborne floras, consisted of comparisons with geographically remote floras like those of the Green River beds of Wyoming and those of the Paris basin and southern England. There are certain features, however, notably a marked indication of a rise in temperatures and increased humidity, which, in so far as they are known, characterize middle eocene floras everywhere. This is known to be the case in America, it is markedly shown in the floras of England and France, and is emphasized in the numerous late eocene floras from a large number of arctic localities.

Among the forms collected from the Georgia Eocene are new species of *Acrostichum*, *Arundo*, *Castanea*, *Conocarpus*, *Dodonaea*, *Ficus*, *Malapoenna*, *Momisia*, *Pisonia*, *Potamogeton*, *Rhizophora*, *Sapindus*, *Terminalia*, and *Thrinax*, including the first fossil occurrence of species of *Conocarpus*, *Momisia*, and *Thrinax*.

The foregoing forms represent twelve families and include one fern, three monocotyledons, and ten dicotyledons. No gymnosperms, which are usually represented in European Lutetien floras by at least the genus *Podocarpus*, have been discovered. It will be observed that only two forms, the *Castanea* and the *Potamogeton*, are not coastal forms, and the latter is an aquatic whose presence associated with coastal swamp or strand plants is not difficult to explain. The *Castanea* then apparently represents the only upland type preserved in this flora, and as it is not common, the presumption is strong that it was brought down to the basin of sedimentation by some eocene river, most likely by the river which originally occupied the trough of the subsequent estuary.

Turning to the remaining twelve species, we may enumerate with profit their closest allies in the existing flora. The *Acrostichum* is represented by *A. aureum* Linn., the *Arundo* by *A. Donax* Linn., the *Conocarpus* by *C. erectus* Linn., the *Dodonaea* by *D. viscosa* Linn.,

the *Ficus* by various tropical American figs, the *Malapoenna* by *M. geniculata* (Walt.) Coulter, the *Momisia* by *M. aculeata* (Sw.) Kl., the *Pisonia* by *P. macranocarpa* Donnell Smith, the *Rhizophora* by *R. Mangle* Linn., the *Sapindus* by *S. saponaria* Linn., the *Terminalia* by *T. phaeocarpa* Eichler, and the *Thrinax* by various West Indian species of that genus.

When we consider the habitat of these modern forms and that of their allies, we find that they inhabit the tidal nipa swamps of the orient, the mangrove swamps of the orient and the occident, the beach jungle of the strand, or the landward side of coastal sand dunes in the tropics. Nearly every one of the fossil species is represented by forms found in the existing flora of the Florida keys or along the shores of peninsular Florida, some like *Conocarpus* flourishing equally well on either muddy or sandy shores. Every species (except *Castanea*) is represented in the American tropics, and four of these existing representatives (*Conocarpus*, *Dodonaea*, *Rhizophora*, and *Sapindus*) range northward to Bermuda in the path of the gulf stream. In looking over SCHIMPER'S classical *Indo-malayan strand flora*, the following forms, which are strictly comparable to the Georgia eocene plants, were noted as being more or less prominent elements in the oriental strand flora: *Acrostichum* (*Chrysodium*, 1 sp.), *Dodonaea* (1 sp.), *Eugenia* (2 species which are represented in the west African and American tropics by the allied genus *Conocarpus*), *Ficus* (1 sp.), *Malapoenna* (*Litsaea*, 1 sp.), *Pisonia* (4 sp.), *Rhizophora* (2 sp.), *Sapindus* (1 sp.), and *Terminalia* (1 sp.). Of these forms the *Sapindus*, *Terminalia*, *Dodonaea*, *Ficus*, *Malapoenna*, and *Pisonia* are more particularly elements of the littoral forest (beach jungle of KURZ, *Barringtonia* formation of SCHIMPER), while the others are integral members or rather intimately associated with the mangrove or the nipa associations. It is really remarkable to what an extent the identified elements in the Claiborne flora corroborate one another and definitely denote the character of their habitats, which were, on the one hand, mangrove swamps at certain points where the conditions were favorable, and elsewhere the vegetation of the rain forest which clothed the sandy beaches or was developed behind dunes, which possibly formed the highest inner margin of the beach in places.

Certain rather definite climatic deductions appear to be justifiable from the foregoing facts. None of the modern representatives of this eocene strand flora flourish north of the winter isotherm of about 50° F., and the majority do not occur north of the winter isotherm of 60° F. None of the fossil forms, except possibly the *Potamogeton*, the modern species of which range over a great many degrees of latitude, or the *Castanea*, which likewise has a wide range, would be expected to occur outside of the areas where the modern subtropical rain forests are developed. We would expect the Claiborne climate in the area under discussion, at least at low elevations along the coast and in proximity to the eocene gulf stream, to have been uniformly humid with an abundant and evenly distributed rainfall. The temperature would have been uniform, not necessarily extremely hot, and any degree of winter cold would have been fatal.

These considerations are in a large measure corroborated by what we find to have been the conditions in Europe at this time. It is well known that the middle eocene floras of Europe show many tropical characters absent in the earlier Eocene. These first become marked in the fruits from the London clays and the leaves from Alum Bay and in homotaxial deposits on the continent, and while it was once the fashion to see Australian affinities in these floras, they show closer affinities with the modern floras of Malaysia and tropical America. The accompanying sketch map (fig. 1) will bring this out very well. It shows the area of distribution of the modern genus *Nipa* and the eocene genus *Nipadites*, which is indistinguishable from the modern *Nipa*. The latter has a single species inhabiting the tidal waters of the Indian Ocean, ranging from India through the Malay Archipelago to the Philippines, and vying with the mangroves for possession of the tidal flats. It produces clusters of large floating fruits, and it is exactly similar in both form and structure to the fossil fruits which form the basis for the genus *Nipadites*. As the map shows, these tropical or subtropical floras ranged northward in Europe at this time to southern England, or to about the latitude of Newfoundland on this continent, and these eocene *Nipa* swamps furnished a congenial habitat for one or more species of *Acrostichum* closely allied to that

of Georgia, and other forms are not wanting. In the Paris basin a species of *Pandanus* is associated with the *Nipadites*, while at Bournemouth *Gleichenia* and various tropical Polypodiaceae occur. The Lutetien stage of the Eocene in Europe and the Claiborne stage in America are both characterized by a remarkable transgression of the sea, the approximate shore line of the Claiborne sea being shown on the accompanying map (fig. 2). This map makes no pretence to exactness, which would be impossible on so small

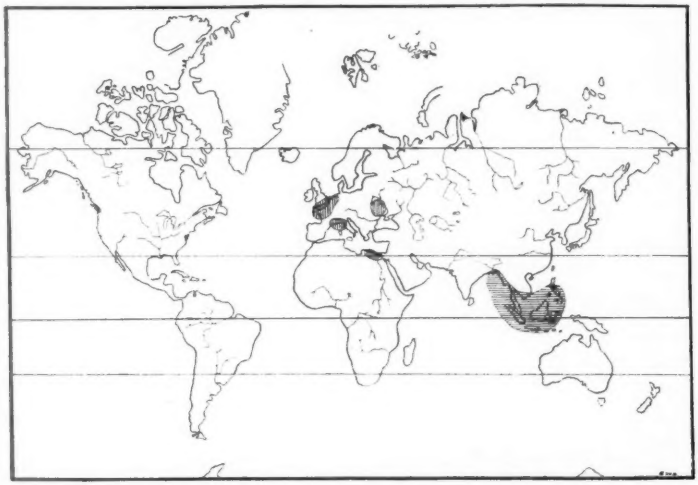

FIG. 1.—Sketch map showing geographical distribution of the existing genus *Nipa* Thunberg and the eocene genus *Nipadites* Bowerbank; horizontally lined area shows single existing species; vertically lined area shows eocene species.

a scale map, even if the areal extent of the Claiborne deposits were accurately known. What it is intended to illustrate is the relation of land to sea and the line of advance of the flora which was necessarily along the shore from equatorial America, where undoubtedly the representatives of this flora flourished during the whole of the Tertiary.

In studying the Georgia flora, the writer became much interested in the means of dissemination of its modern allies, which in so far as they are known are worthy of enumeration. It has been shown that certain species of *Eugenia*, *Terminalia*, *Rhizophora*,

Pisonia, and *Sapindus* have become adapted for dispersal through the agency of ocean currents, by specialization of their fruits or seeds, which have developed air chambers or woody husks for buoyancy, and practically impervious seed coverings for the exclusion of sea water from their vital parts. Everyone is familiar

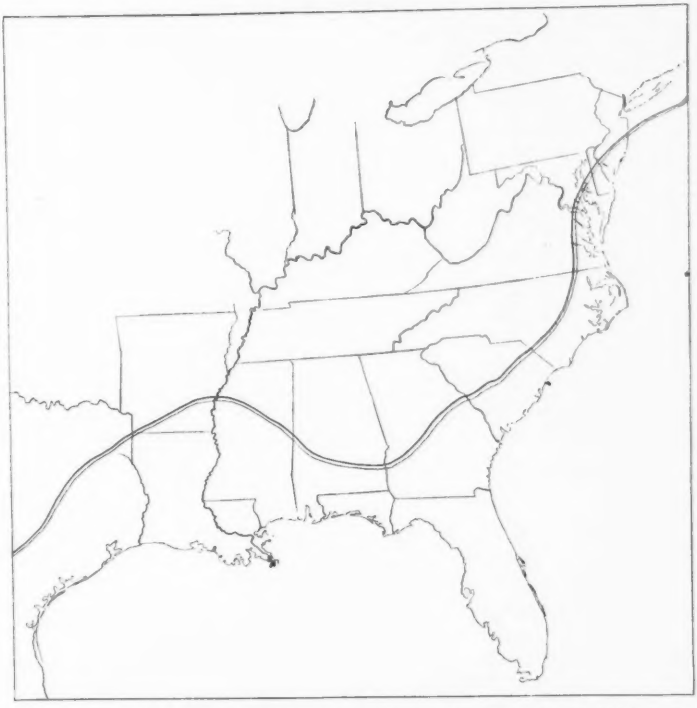

FIG. 2.—Sketch map showing the approximate shore line of the middle eocene sea in southeastern North America.

with the extreme specialization of the mangrove, which sends its germinating plantlets out into the world fully prepared to anchor themselves in water of the required depth, but the comparable specializations of the other members of these floras, though less in degree, are almost as effective, judging by the present geographical distribution of these genera, so that ocean currents must play a considerable rôle in distribution, despite the contrary opinion of DE

CANDOLLE. Other members of the Claiborne flora may be supposed to have been distributed by fruit-eating birds. This seems clearly to apply to *Ficus* and probably to *Malapoenna*.

It can be readily shown that the existing flora of peninsular Florida, the Bahamas, Bermuda, etc., contains a large element which has been derived in comparatively recent geological times from the south. In the case of the Bahamas and Bermuda, almost their entire flora has had such an origin. If, however, we study geographical distribution in the light of historical geology, we find that the main elements of these modern floras were already in existence in the Middle Eocene, if not earlier. Experience teaches us that nearly all modern plant families, unless it be the most specialized forms like the orchids among the monocotyledons or the composites and their allies among the dicotyledons, were at some time more widely distributed than they are at present, and that the details of modern geographical distribution represent in a less degree the interchange of types between different areas than they do the greater or less degree of segregation of descendants of forms once spread over much wider areas. Uniformity appears to have been the rule during geologic history and not the exception.

From a study of the Claiborne flora it is evident that the main elements of the modern flora of tropical America reached as far northward in the Eocene as latitude 33 and probably much farther, and that in post-Eocene time they retreated southward. DALL,[1] from a study of the tertiary faunas of Florida, places a marked change in climate at the close of the Oligocene, and accounts for it by the elevation in the Florida area and the shifting to the eastward of the gulf stream with an inshore southerly flowing cold current.

Thus while the strictly modern movement of the subtropical flora along the course of the gulf stream has been from the south northward as the various coral islands of the Bahamas became evolved, this dispersion was preceded by a similar spread of the tropical flora on a much more extended scale during the early Tertiary.

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[1] DALL, Trans. Wagner Inst. 3:1549, 1550. 1903.

NOTES ON THE SEX OF THE GAMETOPHYTE OF ONOCLEA STRUTHIOPTERIS

DAVID M. MOTTIER

During the past two or three years the writer has been collecting data from a study upon the gametophyte of *Onoclea Struthiopteris* with reference especially to the dioecious character of the prothallia, and while this study is in no way completed, it has been deemed advisable to make public some of the facts observed, inasmuch as the data obtained supplement those set forth in a very interesting paper, appearing in a recent number of this journal, by Miss WUIST.[1]

The impression is general that the prothallia of *Onoclea Struthiopteris* are dioecious,[2] and although this is generally true, it is not strictly so, as bisexual prothallia may readily be found under ordinary and perhaps optimum cultural conditions. In 1907, in an address before the Indiana Academy of Science,[3] I made the following statement:

> The writer has recently begun the study of a fern, whose prothallia have been reported as strictly dioecious, and if the spores of the same are well nourished, female prothallia will predominate, while with poor nourishment the vast majority of spores will give rise to male gametophytes. An examination of cultures grown under favorable conditions for laboratory use, in which the spores were sown thickly, showed that certain spores produced strictly male plants, others female, and still others bisexual prothallia. A small number of spores were isolated and grown under similar and very favorable conditions, with similar results. The pure males were almost equal in number to those bearing the female organs, while the bisexual plants were few, being about 4 per cent of the whole number. The foregoing results seem to lend encouragement to the view that environmental conditions may have much less to do with the development of male and female prothallia than had hitherto been supposed. The very brief study showed clearly that in the fern in question

[1] WUIST, ELIZABETH DOROTHY, The physiological conditions for the development of monoecious prothallia in *Onoclea Struthiopteris*. BOT. GAZETTE **49**:216–219. 1910.

[2] CAMPBELL, D. H., Mosses and ferns. Edition 2, p. 314. 1905.

[3] MOTTIER, D. M., History and control of sex. Proc. Ind. Acad. Sci. 1907. pp. 44–45.

there is a great mortality among the spores, which, as can be readily seen, vary greatly in size. Among the first things to establish in this and similar cases, is whether mortality is greatest among the smaller or larger spores, and whether the prothallia springing from the smaller spores tend to remain small and produce only antheridia, while the larger female plants arise only from the larger spores, and so on.

The fern in question was *Onoclea Struthiopteris*, although the name of the plant was not stated in the address in question.

Miss WUIST (*op. cit.* p. 217) states that about 1 per cent of the prothallia of soil cultures were monoecious. This estimate seems to me to be much too low, inasmuch as an examination of a larger number of prothallia, since the publication of the paragraph just quoted, showed that about 12 per cent were monoecious. The prothallia from which this estimate was made were grown upon soil under good cultural conditions. The plants, although grown thickly, were vigorous, normal in every way, and in fact the cultures seemed to leave nothing to be desired. The monoecious prothallia were as a rule young, bearing one to several archegonia, and were 1.5–3 mm. in diameter. Antheridia were very rarely found on older and larger prothallia, although little attention was paid to exact size and measurement. The antheridia were as a rule upon the ventral side, among or near the rhizoids, hence upon the older tissue; very rarely did they appear at the margin of the prothallium. In one case an antheridium was found upon the archegonial meristem.

In determining whether prothallia were monoecious, I did not rely at all upon an examination of fresh material, but all plants were carefully fixed, stained *in toto* with borax carmine, cleared, and examined in clove oil or balsam. In this way a single small antheridium could easily be found upon a prothallium, and cases were not infrequent of a single antheridium appearing inconspicuously among the rhizoids. It has been my experience that one or two immature antheridia among the rhizoids are easily overlooked when the plants are examined alive. In material thus stained and cleared, one may also more readily and accurately detect very small male plants adhering among the rhizoids of the larger prothallia bearing archegonia.

In order to ascertain whether crowding was in any way respon-

sible for the sex of the gametophytes, cultures were made by sowing spores thinly upon the soil and keeping the cultures under the most favorable conditions available. One such culture may be mentioned in detail. In this 207 spores were sown and 150 prothallia harvested. Of these 78 were pure males, while 72 bore archegonia, and of the latter 10 per cent were monoecious. Cultures were also made to determine, if possible, whether the spores of any given sporangium were male or female, as has been reported for certain liverworts. In this set of experiments the spores of individual sporangia were sown upon earth in separate dishes, with results similar to those from spores sown from many sporangia, that is, both male and female prothallia were grown; of the female, those bearing archegonia, some few were bisexual. Such experiments show conclusively that sporangia do not bear spores giving rise exclusively to male or female prothallia, and the fact that prothallia are bisexual is proof that no sex-differentiating chromosome is present in this fern.

No effort has been made up to this time by the writer to induce the development of antheridia upon "female" prothallia. Interesting experiments along this line have been carried out by Miss WUIST, as mentioned in the foregoing, with the following results: monoecious prothallia were obtained by transferring the plants from distilled water to Knop's solution, in which they were further cultivated; by transferring "female" prothallia from soil to a nutritive solution, and by transferring from one nutritive solution to another.

These experiments indicate that gametophytes bearing archegonia only may be induced to develop antheridia as a response to environmental conditions. Knowing that dioecism has not been completely established, these results, though important, are not surprising. They do not show a very far-reaching influence of environmental factors in regard to the regulation of the development of sex cells; for I have frequently observed in older cultures, that is, about five months after sowing, that certain "female" prothallia, in which an egg failed to be fertilized, developed from the oldest parts numerous small lobes which give that end of the prothallium a finely fringed appearance, and upon these lobes

numerous antheridia were produced. Such prothallia were well nourished, with a deep-green color, and continued to develop the archegonial meristem, which produced continuously numerous archegonia. The specimens before me at this writing are 5–6 mm. in length. As stated, the antheridial lobes were confined generally to the posterior or older parts of the prothallia, but they may extend a short distance forward along the margins. Antheridia were not observed, except in very rare cases, upon the archegonial meristem.

After all, the fact that archegonial plants may develop organs of the opposite sex, either normally or in response to conditions controlled in a measure by the experimenter, may readily find a satisfactory explanation, but the case may be different in regard to the prothallium that produces only antheridia. If purely male prothallia of *Onoclea Struthiopteris* are merely those that do not become large enough, because of nourishment, to bear archegonia, why is it that these pure males cannot be made to grow large enough to develop the female organ? The writer knows of no case on record in which this has been done. If the male plants could be made to develop the archegonial meristem and its archegonia, then it may be said that the environment (which of coures means chiefly nutrition) controls sex to that degree. The writer has not been able to believe that male prothallia of the fern in question are merely those that do not become large enough to bear archegonia, but that certain spores are predetermined to develop into purely antheridial plants, and others into those capable of forming archegonia. If there were a sex-determining chromosome, then one half of the spores would be male and one half female, but as the "female" prothallia bear occasionally antheridia, it cannot be said that a sex-determining chromosome is present. The explanation which we are able to give at present is that in certain spores the male tendency is dominant, while in others it is the female, but such statements mean little at present.

In a foregoing paragraph reference was made to the mortality among the spores, and to the variation in their size and apparent vigor. Some are larger and more healthy looking than others, and, as in many plants, many spores seem to be and doubtless are

abortive. It was thought at one time in this study that the smaller spores were the ones that produced the purely antheridial prothallia, but sowings were made by selecting, in so far as possible, the more vigorous spores (an extremely tedious process), but the results did not bear out the expectation. Owing to the difficulties of selecting individual spores, it is not maintained that the last-named experiment is of any great value, at least the writer does not lay any stress upon the results. It seems reasonable that a larger percentage of the smaller spores, that is, those which show less chlorophyll and which germinate less readily, would fail to grow than in the case of the larger ones, so that the possibility of the smaller and abortive-looking spores producing chiefly male prothallia is not excluded. However, on this point the writer can make no definite assertions.

The foregoing statements may be summarized as follows:

1. The spores of *Onoclea Struthiopteris* when grown upon earth, under optimum cultural conditions, produce regularly three kinds of prothallia: small plants bearing only antheridia, the so-called male gametophytes; larger prothallia bearing only archegonia, the female gametophytes; and those bearing both archegonia and antheridia, the bisexual or monoecious prothallia.

2. Archegonial prothallia, which continue growth without bearing a sporophyte, sometimes develop numerous small lobes from the older portions, upon which numerous antheridia appear.

3. The gametophyte, therefore, is not strictly dioecious, and there is in all probability no sex-determining chromosome.

4. It is highly probable that the development of purely male or female gametophytes is not dependent upon conditions of nutrition, but that the sexual tendency is predetermined in the spore. Environmental conditions, or the failure of an egg to give rise to a sporophyte, owing to a lack of fecundation, may induce the development of antheridia upon archegonial plants, which continue their growth for some months.

5. Pure males result, in so far as is known, under good cultural conditions from the dominance of the male tendency in the spore over the female tendency.

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BRIEFER ARTICLES

AN ATMOGRAPH

(WITH FOUR FIGURES)

The introduction of the porous cup atmometer by LIVINGSTON has been followed by great activity in securing evaporation data in relation to plant life. Readings have usually been made with reference to a zero point, usually a file-scratch, on the wall of the reservoir containing the water. For most workers it has been impossible to visit the field more frequently than once a week. The records secured were of course simply the total weekly evaporation.

It is becoming increasingly clear that the record of total weekly evaporation can be evaluated only when we know the rate of evaporation at all times, and particularly when we know the maximum and minimum rate. This need can be met only by some form of automatic measuring and recording device. No such instrument has yet come into general use. To meet the conditions of the work such an instrument must measure in convenient units and record in graphic form. It must be so simple that it may be set up easily in the field and trusted to work with only occasional attention. Also the cost of construction must not be so great as to prohibit the employment of a sufficient number of them to secure comparative data. In attempting to meet these conditions as I have found them in the field, the instrument described herewith has been designed (fig. 1).

The device (fig. 2) consists essentially of an oscillating beam (A), which may be caused to tilt by the movement of mercury in a tube (B) and its connected bulbs, advantage being taken of the oscillation to open the stopcock (C) and to close it again. At the same time an electric circuit is closed which actuates the pen of the chronograph.

In practice the whole apparatus is filled with water and the stopcock closed. Mercury is poured into the bulb b', nearly filling it, the inlet e' is connected with the supply tank of water, and the outlet at d' with the atmometer. The water which evaporates from the atmometer is replaced by a flow through the pipes d, c, and a from the supply in the bulb b'. The mercury in b follows the water, partially filling b'.

Whenever the transfer of weight from b to b' has gone far enough to shift the center of gravity to the right of the center of suspension

(F), the beam A is caused to revolve until checked by the stop g'. As the end of A moves upward it carries with it the flange f, which strikes the

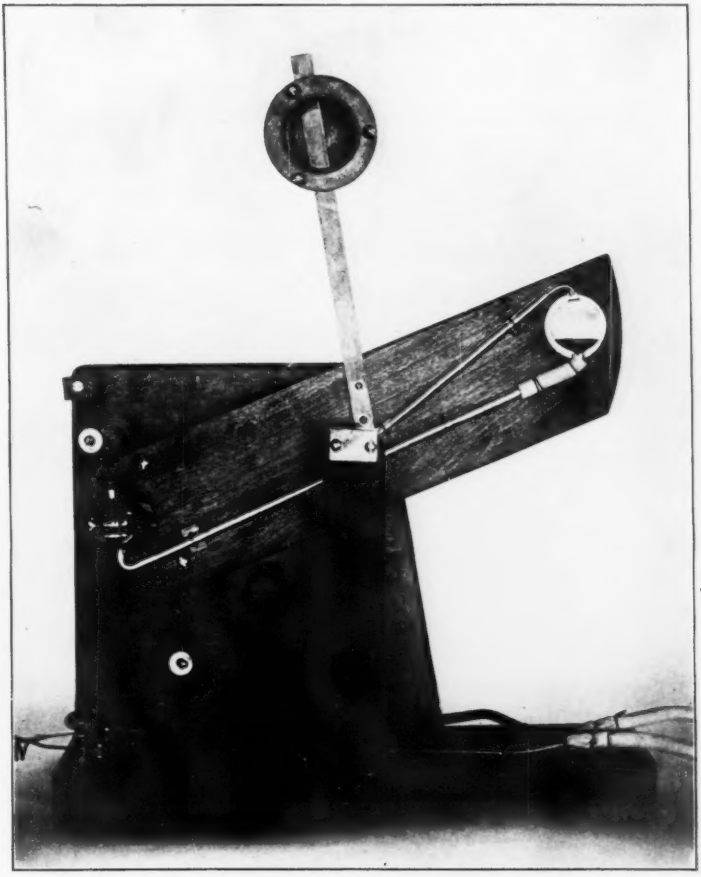

FIG. 1

handle of the stopcock and pushes it upward, thus opening the passage from the water supply through e, c, and a into b'. The stop g' is so located that b does not rise to quite so high a level as b', and the mercury

therefore flows back toward b, followed by the water which again fills b'. The shifting of weight toward the left causes A to return to its

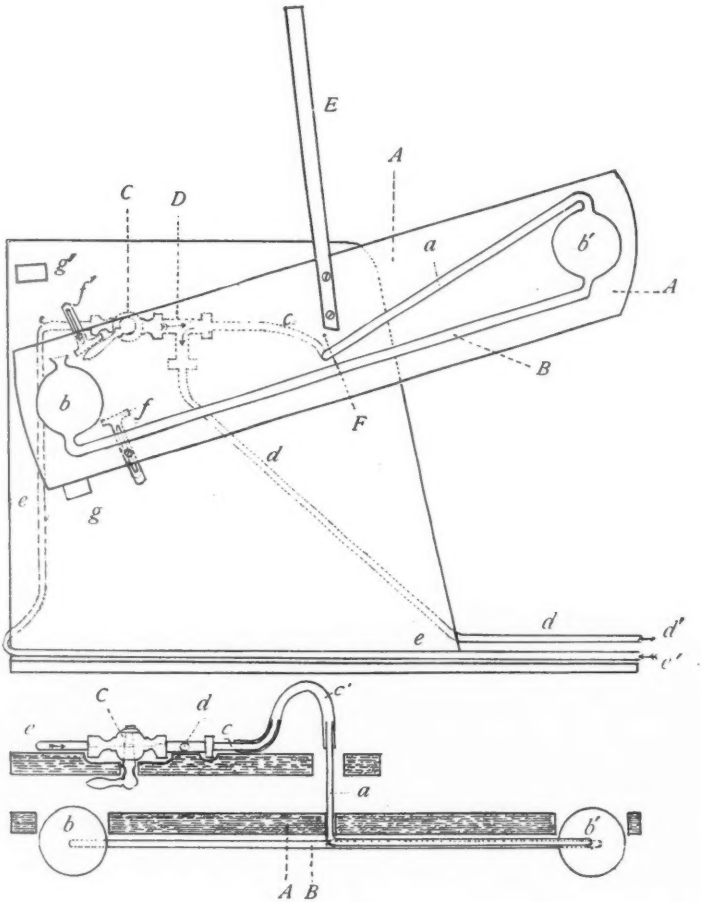

FIG. 2

former position, the flange f' in its downward movement closing the stopcock C.

The point of suspension (F) is placed lower than the center of gravity

of the whole system consisting of A and its attached parts. In the initial position, as shown in the figures, the center of gravity is not only higher than F, but also at some distance to the left of a perpendicular through F. A considerable amount of mercury must therefore pass into b' before the center of gravity will have shifted to the right of the perpendicular sufficiently to tilt the beam. The tilting of the beam carries the center of gravity yet farther to the right, and the beam therefore rests in the new position until the reverse movement of the mercury has carried the center of gravity to its former position in the beam. It is evident that if the center of gravity be raised yet higher above F, it will also be farther to the left when in the first position, and a larger volume

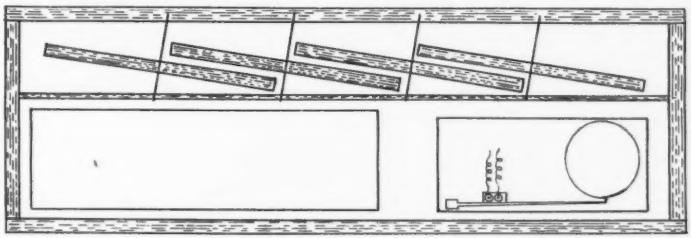

FIG. 3

of water will have to be displaced from b' by mercury before the beam will tilt. Each record, therefore, will indicate a larger amount of water.

Advantage is taken of the relation between the center of gravity and the center of suspension to calibrate the instrument. A weight is placed upon the bar E, and by sliding it up or down the bar or exchanging it for another, the center of gravity is adjusted at will. The point of suspension may also be moved.

The atmograph, set up as shown in the photograph (fig. 4), recorded the passage of 10 cc. units. By lowering the weight, the quantity could be reduced by half, or set at any intervening amount. Experience indicates that under ordinary conditions 10 cc. will be a convenient unit for field work with LIVINGSTON atmometers.

Standard copper pipe and fittings are used throughout, no rubber being employed excepting for the flexible connection c'. Electrical connections are made from one of the binding posts shown in the photograph to the pipe e, and from the other to the flange f. The circuit is closed through the stopcock while f is in contact with the handle. A single dry cell is employed as the source of current.

The atmograph may be employed in the measurement of the transpiration of an excised branch by tying the branch into a rubber tube and connecting with the outlet at *d'*, or it may be used in other cases in which it is desired to measure the passage of water.

For observation in the field the instrument must be inclosed in a box in order to preserve it from interference. If it is desirable to operate several atmometers at a single station, as described by YAPP, the atmographs may be compactly arranged in a box with a common reservoir

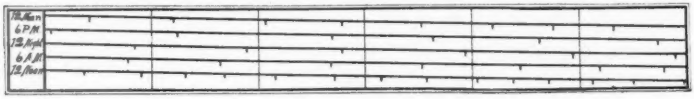

FIG. 4

and a single chronograph (fig. 3), as many pens being used as atmographs. Experience has shown that the piping leading to the atmometers should be metallic. Glass and rubber are subject to many hazards, not only from vandals but also from the less conspicuous members of the fauna. Grasshoppers sometimes exhibit a very interesting, but none the less disastrous, adaptation of appetite to the supply of rubber tubing.

For a series of simultaneous records a chronograph drum rotating in seven days will be found desirable. The records shown in fig. 4 were made in the laboratory upon a chronograph rotating once in six hours and with a group of four LIVINGSTON atmometers. The interval required for the evaporation of 10 cc. by the four cups has been noted to vary, within twelve hours, from a maximum of 113 minutes to a minimum of 17 minutes.—W. L. EIKENBERRY, *The University of Chicago.*

THE SPORANGIUM OF LYCOPODIUM PITHYOIDES

(WITH PLATE VII)

So much importance has been attached at various times to the position, shape, and development of the sporangium in *Lycopodium*, that any unusual forms are of interest. The rare species *L. pithyoides* Schlect and Cham., which is figured in a former paper,[1] presents certain variations. The stem tip is large and blunt, with a flattened apical region. The earliest stages in the development of the sporangium agree with those of previously

[1] STOKEY, A. G., The roots of *Lycopodium pithyoides*. BOT. GAZETTE **44**:57–63. 1907.

described species (fig. 6). The leaves appear in close succession on the flattened tip, leaving no evident internodal region. The development of the internode afterward is very rapid, causing a conspicuous shifting of the position of the sporangium (fig. 7). The rapid growth of the internode, combined with the unequal rate of growth of the two sides of the sporangium, causes the foliar structure to become axial. A change of position to this extent is not uncommon in other species of *Lycopodium*, but in *L. pithyoides* there is a continued inequality in the rate of growth, so that the sporangium eventually takes a position on the stem entirely distinct from the leaf. At maturity the sporangium is not disturbed if the leaves are pulled off. The development in this case is the reverse of that in most species of *Selaginella*, in which the sporangium arises as a cauline structure, but becomes foliar when mature; while in *L. pithyoides* it is foliar in origin, but cauline at maturity.

The sporangia of *L. pithyoides* are very large, attaining a breadth of 2–2.5 mm., and resemble those of *L. dichotomum* Jacq., according to BOWER's description and comparison,[2] although Miss SYKES's drawing,[3] while suggesting a tendency in the sporangium to be cauline, does not indicate a very close resemblance in other points. The stalk is short and relatively slender, ranging from 12 to 18 cells in diameter in sections cut radially, and about twice that in the other diameter. The cells are elongated and thin-walled, with no corner thickening and no trace of lignification such as Miss SYKES has described in *L. clavatum* and other species. The vascular strand of the leaf shows no tendency to approach the sporangium. The leaf trace arises from the vascular cylinder 5–6 mm. below the sporangium, turning sharply upward and then making an outward bend as it approaches the leaf.

The sporangia resemble those of *L. dichotomum* in the number of wall layers also. In the upper part of the sporangium there are usually four layers, but there may be even more owing to the irregularity in the arrangement of the middle layers (fig. 4). At the base of the sporangium the wall may consist of 6–8 layers. In a young sporangium (fig. 8) the order of division and the relation of the layers is very plainly seen, but in the older sporangia this regularity of arrangement is lost. The inner layer or tapetum is well defined as a dense, darkly staining layer. The tapetal cells do not show any tendency to become rounded on the inner face. In

[2] BOWER, F. O., Studies in the morphology of spore-producing members; Equisetineae and Lycopodineae. Phil. Trans. Roy. Soc. London B **185**:516. 1894.

[3] SYKES, M. G., Notes on the morphology of the sporangium-bearing organs of the Lycopodiaceae. New Phytologist **7**:41–60. 1908.

some almost mature sporangia the tapetum is alive, while in others very little older the tapetum is entirely empty, but granules and droplets are abundant along its inner face. The emptying process is apparently a rapid one, as a considerable number of sporangia were found in both conditions but none in any intermediate stages. The cells of the tapetum retain their shape for a considerable time and do not become crushed and flattened until much later. The subarchesporial pod is well developed (fig. 2) and slightly irregular in outline, but does not form processes in the sporangium.

The leaves afford little protection to the sporangia except in the very early stages, owing to the fact that they are relatively narrow (figs. 3, 5) and soon become recurved. The line of dehiscence, which is marked by smaller cells than the rest of the wall, is median (figs. 1, 3).—ALMA G. STOKEY, *Mt. Holyoke College, South Hadley, Mass.*

EXPLANATION OF PLATE VII

FIG. 1.—Longitudinal section of sporangium. ×28.

FIG. 2.—Tangential section of a sporangium. ×26.

FIG. 3.—Transverse section through base of sporangium and sporangium stalk. ×28.

FIG. 4.—Section of part of the wall of sporangium. ×345.

FIG. 5.—Transverse section of sporangium. ×28.

FIG. 6.—Longitudinal section through leaf, showing one of the initial cells of the sporangium. ×725.

FIG. 7.—Longitudinal section through stem tip, showing three stages in development of sporangia. ×36.

FIG. 8.—Longitudinal section of young sporangium, showing origin of wall layers ×345.

THE BOTANICAL CONGRESS AT BRUSSELS

The Third International Botanical Congress was held at Brussels, Belgium, May 14–22, 1910. Saturday, the 14th, was the day for registration. On Sunday, the 15th, the members of the congress assisted at a session of the Royal Botanical Society of Belgium held in the "dome" of the large building connected with the Jardin Botanique, at which several interesting papers were presented by the members of the society. Two of the general sessions of the congress were held in the same room, the opening session on Monday morning the 16th, and the closing one on Sunday the 22d. No regular sessions of the congress were held in the evenings, but during the week several interesting papers on phytogeographical subjects, economic botany, etc., were given in the evening.

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Of the three sections into which the congress was divided for the special work of the week, perhaps the most important was the "Section on nomenclature." The meetings of this section were held on the Exposition grounds in Festival Hall.

As is well known, the Vienna Congress in 1905 selected LINNAEUS' *Species plantarum* (1753) as the starting point for the nomenclature of the seed plants (Spermatophytes) and vascular cryptogams (Pteridophytes). It also established the general principles and codified the rules which form the *rules of nomenclature for plants*. In dealing with the "cellular cryptogams" certain problems were presented which the Vienna Congress decided should have special consideration, namely the question of different, later starting points for the nomenclature of different groups of the "cellular cryptogams," and the problems connected with the nomenclature of the fungi possessing a pleomorphic life cycle.

As to the starting point for the nomenclature of plants, it is well known that there were two opinions, as follows: (1) that there should be a single date recognized for the beginning of the nomenclature of all plants; this opinion was based on the principle of uniformity in time or date as the starting point; (2) that there might be several different (multiple) dates or starting points for the nomenclature of different groups; this opinion was based on the principle that *uniformity in the selection of the earliest comprehensive work treating a group, large or small, in a somewhat modern sense*, was of more importance than the principle of uniformity of date. Therefore the Vienna Congress wisely decided to refer the consideration of the nomenclature of the "cellular cryptogams" to the Brussels Congress in 1910, in order that these problems might be studied in the meantime.

Since it will be several months before the complete proceedings of the Brussels Congress can be published, we present here, for the benefit of American botanists, a brief statement of the most important legislation enacted by the "Section on nomenclature."

On Tuesday, the section began the consideration of the motions relating to the nomenclature of the "cellular cryptogams," and, with the exception of Thursday, which was devoted wholly to excursions, the work, together with the motions relating to paleobotany and phytogeography, was continued throughout the week. The different groups were taken up in the order in which they were presented in the preliminary publication, including the various motions, the result of the preliminary voting by the special commissions, the comments of the *rapporteur général*, and the provisional draft of rules. In general session of the section it was voted to postpone the consideration of the bacteria, diatoms, and flagellates, and to take

LINNAEUS' *Species plantarum* (1753) as the starting point for the nomenclature of the Myxomycetes.

At the opening of the session after the noon recess it was suggested that an adjournment of the session be taken for an hour in order to allow all the specialists in the different groups of cryptogams who were present to hold an informal conference for the purpose of agreeing upon recommendations as to the dates for the starting points of nomenclature which would be acceptable to them. These recommendations were presented to the section on Tuesday afternoon and Wednesday morning, and were adopted as rules without further discussion, no one expressing a desire to discuss them in general session. The majorities[4] in favor of the different votes were very large, in the case of fungi, for instance, 130 to 4. The dates adopted by the Brussels Congress, therefore, for the starting points for the nomenclature of the "cellular cryptogams" are as follows:

MYXOMYCETES.—LINNAEUS, *Spec. plant.*, 1753.

FUNGI.—FRIES, *Syst. Myc.*, 1821–1832, except for the Uredinales, Ustilaginales, and Gasteromycetes, which date from PERSOON'S *Synopsis*, 1801.

LICHENS.—LINNAEUS, *Spec. plant.*, 1753.

ALGAE.—GOMONT, *Nostocaceae homocysteae*, 1892–1893; BORNET et FLAHAULT, *Nostocaceae heterocysteae*, 1886–1888; RALFS, *British Desmidiaceae*, 1848; HIRN, *Oedogoniaceae*, 1900; LINNEAUS' *Spec. plant.*, 1753, for all other algae except the Chroococcaceae.

BRYOPHYTES.—HEDWIG, *Spec. musc.*, 1801–1830, for the Mosses; LINNAEUS, *Spec. plant.*, 1753, for the Liverworts.

The general position taken by the congress is well shown in the case of bryophytes, in which the fundamental *Species muscorum* of HEDWIG was adopted for mosses; while for liverworts, on which there exists no work corresponding to the *Species muscorum*, it was decided to go back to LINNAEUS.

Action upon the Chroococcaceae, bacteria, diatoms, and flagellates was postponed for future discussion, partly because it was difficult to select satisfactory works as a basis of nomenclature, and partly because those groups have been studied also by zoologists, and it is therefore necessary to take into consideration zoological as well as botanical treatises.

With regard to the nomenclature of the imperfect fungi, the following rule was adopted:

[4] The voting on these questions, and some others, was taken by a count of the number of votes to which each delegate was entitled, and the number of votes cast was therefore greater than the actual number of delegates present. On certain general questions the voting proceeded simply by a show of hands.

FUNGI WITH PLEOMORPHIC LIFE CYCLE.—1. The different successive stages of the fungi with pleomorphic life cycle (*anamorphoses, status*) can bear only a single generic and specific name (*binome*); that is to say, the oldest, from the starting point of the nomenclature of the fungi, which has been applied to the perfect stage, provided that in other respects it conforms to the rules.

2. For the purpose of nomenclature it is agreed that the perfect stage of fungi with pleomorphic life cycle is that which bears the ascus in the Ascomycetes, the basidium in the Basidiomycetes, the teleutospore in the Uredinales, and the spore in the Ustilaginales.

3. Generic or specific names applied to imperfect stages may not be used to replace a name applied to one or more species, any one of which contains the perfect stage.

Citations of pre-Friesian or of pre-Persoonian names follow the rule. Examples: *Boletus edulis* Fries, not *B. edulis* Bull.; *Polyporus ovinus* Fries, not *P. ovinus* (Schaeff.) Fries. Writers who prefer, however, may write *Boletus edulis* Fries ex Bull.; *Polyporus ovinus* Fries ex. Schaeff., etc.

It is recommended in the case of biological species (*formes speciales*) among the rusts that authors who prefer to employ double names take them from the names of the host plants. A recommendation which was offered as applying to the fungi, namely that when a new genus is published, if there are more than one species the author should cite one as the type species, or if only one species, that one to be regarded as the type of the genus, was adopted and made to apply to all plants.

The expression of opinion on the desirability of having extensive lists of *genera conservanda* was so strong that it was practically unanimous, and commissions were appointed to prepare such lists in the fungi, lichens, algae, mosses, and liverworts.

The following action was taken in regard to *genera conservanda* in the Pteridophytes, and additional ones in the Spermatophytes presented for consideration at Brussels. *Selaginella* was placed among the *genera conservanda*, while the remaining genera in the proposed list of Pteridophytes were rejected.

A commission, which had been appointed for the purpose in advance of the congress, carefully considered the additional *genera conservanda* among the Spermatophytes in the list proposed by JANCHEN, and recommended that 21 or 22 names be stricken out. The list as amended, with the addition of the name *Welwitschia*, was adopted.

The motion to amend the Vienna rules by striking out the clause requir-

ing a Latin diagnosis of new genera and species was voted down Monday afternoon, along with several other motions of a general nature. The question was discussed, however, at a later time when considering a motion by the paleobotanists, to the effect that a diagnosis be required only in one of the following languages: French, English, German, or Italian. This discussion broadened into a general one, and although it was defeated the discussion showed that there was a strong sentiment against the Latin requirement, especially on the part of the American botanists, and the subject will probably be brought up again for discussion at the next congress.

With reference to the question of nomenclature in phytogeography, the following principles were adopted, and the commission, considerably enlarged, was continued.

1. Nomenclature is to be avoided, and the expression *terminology* is rather to be employed.

2. When technical words are employed, a clear definition of them should be given in the sense in which the writer uses them, and also where a term is used in a sense different from that in which it has formerly been employed.

3. It is recommended to use terms taken from living (vulgar) languages to designate associations, etc., and reserve expressions of Greek or Latin origin for higher units, where there are rarely equivalents in the living tongues (examples: mesophytic, hydrophytic, etc.).

4. The principle of priority has no legal value in phytogeography. Terminology is very different from nomenclature, and must be subject to change in order to bring it in harmony with the change of ideas in the interpretation of facts.

5. A recommendation for the establishment of clear phytogeographical maps was adopted.

6. Definition of ecology: Phytogeographical ecology is the study of plants and plant associations in their relations with the medium (surrounding medium or environmental conditions).

7. Without giving a definition of the words *formation* and *association*, the section recommends the use of "formation" in a wider ecological sense, and "association" in a more restricted, floristic sense. For example, meadow, prairie, etc., are formations; but an alpine meadow on granitic soil in central Switzerland is an association.

8. It was decided to publish a dictionary of phytogeographic terminology, containing all the pertinent expressions used in phytogeographical and floristic works, with the original definitions and bibliographical references, and their equivalents in English, French, and German.

9. It was proposed that when such words as *zone* and *region* are used in different senses in different countries, to employ new and clear expressions; for example, *étage* (level or floor) = *Höherenregion; Tieferenregion* of the Germans = *zone altière* or *zone abysssale* of the French.

10. A commission was named for the above purpose, consisting of the present members, with many additions, giving the committee power to add still others.

In the publication of the proceedings of the congress, the rules and recommendations adopted at Brussels will be incorporated in their proper places with those adopted at Vienna, the latter being reprinted, so that the rules of nomenclature for plants will be presented in a single and convenient brochure.

The members of the commission on the nomenclature of the cryptogams are greatly indebted to Dr. BRIQUET for summarizing in a comprehensive manner the many different and often conflicting views expressed by the specialists of the commission in their preliminary correspondence. DR. BRIQUET also rendered a most important service in interpreting the remarks made by different speakers in French, English, and German, so that they were understood by all those present. Professor FLAHAULT, Professor MANGIN, and Professor ENGLER, who presided over the different sessions, performed their duties in such a way as to deserve the hearty thanks of all the members, combining a courteous and affable manner with a strictly business management.—W. G. FARLOW and GEO. F. ATKINSON, *Paris, May 28, 1910.*

CURRENT LITERATURE

BOOK REVIEWS

Heredity as an exact science

Problems of evolution have taken a dominant place in biology ever since the appearance of DARWIN'S *Origin of species*. During the latter part of the last century there was little important progress made, though there was an abundance of academic discussion and repeated analysis of data recorded by DARWIN and a few other observers; but already in the closing years of the century a reaction had set in. With the beginning of the present century this reaction reached its first important expression with the appearance of DE VRIES'S *Mutationstheorie*, which marked the beginning of a new epoch in the study of the various factors of evolution, an epoch not inappropriately called the "era of *experimental* evolution." Other branches of biology have exhibited at the same time a corresponding development. Instead of pure speculations, and of generalizations from observations uncontrolled by experimental conditions, there has been a swiftly growing demand for greater exactness in observations and for experimentation.

As an essential part of the reaction, the development of mathematical methods by Professor KARL PEARSON and a few others promised for a time the key to the riddles of evolution. This movement, led by a pure mathematician, developed a series of beautiful methods for the mathematical analysis of data and the comparison of variations. These methods are of the greatest importance when rightly used, but owing to the almost invariable lack of an equally keen *biological* analysis, the applications of these methods have led to a largely spurious product, whose showing of depth and accuracy has been illusive, not because the methods are faulty, but because of their application to data which did not supply the one fundamental assumption of homogeneity upon which the whole biometrical system is based.

Parallel with this movement toward the use of mathematically precise methods, there has been a rapidly increasing utilization of pedigree culture, or genetic methods involved in efforts to distinguish between mutations and fluctuations, and in Mendelian investigations in heredity, which methods lay stress chiefly upon biological analysis. The antagonism of active workers with biometric and genetic methods in the study of heredity scarcely permitted it to be hoped that a work might soon appear which would give a sound and well-balanced treatment of heredity, utilizing the results gained by both biometry and genetics. On the one hand were the mathematical writings of Professor PEARSON and a number of his students, in which mathematical methods are

applied to data without a preliminary biological analysis to determine whether they were indeed homogeneous, as they were assumed to be; on the other hand, the writings of Professor BATESON and many others dealt purely with alternative types so different from each other that they could be classified by ordinary inspection, and ignored entirely the fundamental value, and indeed the necessity, of biometrical methods in the study of less divergent types.

It has remained for Dr. JOHANNSEN[1] of Copenhagen, himself an ardent student of quantitative variation by biometrical methods, harmoniously to combine the mathematical analysis with a biological analysis of his material, that enables him to present a thoroughly well-balanced treatment of heredity and variation in the light of all these recent refinements of method. His book is presented in the form of twenty-five lectures, which give the clearest and simplest discussion of the statistical methods and their exact significance which has yet appeared. His full, elementary, and illuminating explanations of all the biometrical methods, with simple examples showing their usefulness and their limitations, are a noteworthy contribution, since they render an otherwise difficult and to many persons distasteful subject easily intelligible to anyone who will seriously undertake its mastery. While explaining the meaning of all the biometrical methods and their importance in the exact investigation of variation and heredity, the author continually lays stress upon the fact that these methods are instruments whose usefulness depends upon the quality of the data to which they are applied. Much attention is given to irregular, one-sided, or bimodal variation curves, and it is shown that these different types of curves may be produced by any one of several different causes, and that therefore the occurrence of such curves gives no indication of the interpretation which is to be placed upon them. The interpretation must always come by biological analysis.

The biological conception which forms the keynote of the author's entire discussion is *the permanence of the elementary types* which collectively make up all the systematic species of plants and animals. This is essentially the same conception as the "elementary species" of DEVRIES, but by JOHANNSEN it is given an experimental support of such consistency and magnitude as to place it upon a much firmer basis than it has had heretofore. The existence of such permanent types was first given a biometrical demonstration by JOHANNSEN in his *Ueber Erblichkeit in Populationen und in reinen Linien* (1903), and the present work is in large measure an elaboration and application of the conclusions reached in that paper, supported by the results of a number of added generations and additional critical experiments.

The author's keen analysis of the different kinds of types is having a large influence upon present discussions of heredity and evolution. For the elemen-

[1] JOHANNSEN, W., Elemente der exacten Erblichkeitslehre. Deutschen wesentlich erweiterte Ausgabe in fünfundzwanzig Vorlesungen. pp. vi+516. *figs. 31.* Jena: Gustav Fischer. 1909.

tary forms—whether "elementary species" or "varieties" in the DeVriesian sense—he has proposed the convenient term *biotypes*. He also distinguishes them as *genotypes*, that is, collections of individuals having like germinal characters to distinguish them from mixtures of individuals having like external characters, but of unlike germinal composition. To these latter mixtures, whose uniformity is only apparent, he gives the name *phenotypes*.

Heredity is recognized as only one of many factors involved in evolution, and the author does not even attempt to enumerate the other factors, discussing briefly only such other evolutionary factors as are directly related to the question of permanence of types, for example, natural selection, Weismannism, direct influence of environment, inheritance of acquired characters, etc. He dismisses all which are not based upon a clear distinction between the different kinds of variation and the different kinds of types as purely speculative, and therefore as having no place in the exact science of heredity.

The author recognizes that there may be several ways in which new biotypes come into existence, for example, by changes in the characters of the genes or determiners, loss of genes, combinations of different genes through hybridization, etc., but devotes relatively little attention to this phase of the subject.

While less than 100 pages are given to the results of genetic studies in mutation and hybridization, these subjects are treated in a very satisfactory manner. It is impossible in so short a space nearly to cover these fields in detail, and the method used is the same as in the biometrical part of the book, appropriate examples being introduced to illustrate each phase of the subject, no attempt being made to give a comprehensive account of the many investigations which have been made. With a detailed account of the recent work in Mendelian heredity, we are fortunately supplied at the present time by BATESON'S most recent book[2] on the subject, which, together with this book by JOHANNSEN, gives us a practically complete discussion of what has been done in the study of heredity by exact scientific methods.

While JOHANNSEN'S book is written in German, and is therefore less accessible to the entire English-reading public than might be wished, he has fortunately written with great simplicity and directness, and thus his work is more easily read than is often the case. The author has been criticized by German reviewers because of certain mistakes in German idiom, but the slight departures from the pure German idiom always occur in the direction of the Danish idiom, which is more nearly like the English; consequently, his book is more easily read by English readers than it would be if it followed the German idiom more closely. The value of the work is greatly enhanced by the fact that the author's discussions are illustrated at every point by detailed examples from his own incisive experiments. It is, therefore, a treasure-trove of new knowledge, leading to conclusions which are certain to have a large place in the future

[2] BATESON, W., Mendel's principles of heredity. pp. xiv+396. *pls. 6. figs. 37.* Cambridge: University Press. 1909.

discussions of heredity and evolution. The book is worthy of a place beside DARWIN'S *Origin of species* and DEVRIES'S *Mutationstheorie*, and is certain to be rated as a classic example of the new spirit which has entered into biological investigation in the beginning of the twentieth century.—GEO. H. SHULL.

Outlines of bacteriology

The scope of a work on bacteriology by ELLIS[3] is outlined in the following sentence of the Introduction: "This book is intended to serve as an introduction to bacteriology in all its branches, though more attention has been bestowed on that aspect of the subject which is of the most interest to students of technical and agricultural bacteriology."

Bearing the above introductory sentence in mind, the reviewer is the more impressed by the apparent "errors of omission and commission," the lack of facile expression, misstatements of fact, and in places the absence of a knowledge that denotes real intimacy with certain phases of the subject to be presented. And it is marvelous that the firm of Longmans, Green & Co., should have undertaken the publication of the volume.

Without touching upon numerous smaller mistakes and errors, attention may be drawn to the prominence given the author's views upon the supposed very general flagellation of the members of the Coccaceae (pp. 19, 20). It might be well to point out that those views are not yet by any means concurred in by many eminent authorities.

The crudity of the method recommended for the observation of the germination of spores (p. 32) is brought into relief when compared with the superior advantages afforded by the use of the hanging-drop, or the hanging agar-block on the warm stage.

In the light of the best American practice, the method advised for the handling of gelatin petri dish cultures is incomparably cumbersome and unnecessary (p. 47); while the strictures passed upon the use of agar under similar conditions are scarcely warrantable (p. 49).

In his discussion of the effect of the electric current upon bacterial life, it would seem that the author had not read far enough afield, else the statements made by Professor J. BEHRENS in LAFAR'S *Handbuch d. techn. Mykologie* (vol. I, sec. 99, p. 455) would have vastly modified his opinion.

Page 61 gives undue prominence to MELTZER'S ideas regarding the adverse action of moderate degrees of continuous vibration upon bacterial cells. It may be said that those views no longer carry weight.

In the chapter on sterilization (p. 85) are to be found the following remarkable statements in reference to the sterilization of the air of a room: "It may at once be stated that no gas is a disinfectant. . . . . Obviously, therefore, if we wish

[3] ELLIS, DAVID, Outlines of bacteriology (technical and agricultural). 8vo. pp. xii + 262. *figs. 134.* London: Longmans, Green & Co. 1909. *7s. 6d.*

to purify a room, our only chance consists in spraying the walls, the floors, and the objects in the room with a reliable liquid disinfectant. This tends to purify the places from whence the air derives its supply of bacteria." The admirable work of CHICK on the action of disinfectants receives no comment, whereas MIQUEL'S work, so long out of date, is given great prominence.

In the chapter on sewage and sewage disposal (p. 239) are to be found remarks that in this country at least would be deemed erroneous, improper, and inadvisable. For instance, who would willingly subscribe to the following: "There is great similarity between *Bac. typhosus* and *Bac. coli communis*, an organism which is very common in sewage, and which is strongly suspected of being the cause of epidemic diarrhea, though positive proof is still wanting"? Dr. ELLIS condemns the methods of killing off bacteria in sewage by antiseptics as "not practicable," whereas RIDEAL in his own country, and PHELPS and CARPENTER and others in the United States, have shown how valuable an agent is calcium hypochlorite in this direction.

In the section heading "Disposal without purification" (p. 247), discussing the disposal of the contents of cesspools, the author writes: "The other [insoluble] substances that in larger places usually find their way to the sewage drain are thrown broadcast on to any convenient spot, such as a roadside or a neighboring common. This method is efficient enough for very small places, though it must detract somewhat from the healthiness of village life." It is to be regretted that Dr. ELLIS puts himself on record as condoning such a practice; no matter under what conditions of life, the practice is sufficiently vile and unsanitary to be most strongly condemned.

It is very noticeable that no chapter on the biological methods of water purification found a place in the book, despite its importance to technical students.

One cannot turn over the pages of the book without remarking upon the inexcusable crudeness of some of the drawings, which actually mar what otherwise makes a most presentable volume.—NORMAN MACL. HARRIS.

Vegetable proteins

Another of the monographs on biochemistry edited by PLIMMER and HOPKINS has just appeared,[4] and deals with vegetable proteins. It is hardly necessary to state to biological chemists or plant physiologists that no one can speak more authoritatively upon this topic than OSBORNE. Plant physiologists, of whom a fundamental knowledge in several accessory sciences is demanded, are sure to welcome a work of this kind. It shows directness and force that comes from the author, who is the greatest producer in the subject he is discussing. The bibliography consists of 608 citations.

A list of the chapter headings will give a good idea of the scope of the work: historical review; occurrence of proteins in different parts of plants and their

[4] OSBORNE, THOMAS B., The vegetable proteins. 8vo. pp. xiii + 125. New York: Longmans, Green & Co. 1909.

general characteristics; isolation and preparation of seed proteins; basic and acid properties of proteins; solubility of vegetable proteins; precipitation of vegetable proteins; denaturing of vegetable proteins; physical constants of vegetable proteins; products of hydrolysis of vegetable proteins; classification of vegetable proteins; some physiological relations of vegetable proteins to the animal organsim and the biological relations of seed proteins to one another.

The plant physiologist will welcome this work expecially, for most discussions of proteins deal in the main with animal proteins.—WILLIAM CROCKER.

MINOR NOTICES

A new catalogue of Connecticut plants.—The Connecticut Botanical Society, through a committee of six of its members, has issued recently a *Catalogue of the flowering plants and ferns of Connecticut.*[5] The publication has been modestly termed a *catalogue*, but it is far more than a mere list of plants of the state. The scientific and common names of the plant are given, as well as limited synonomy, habitat, distribution, and citation of exsiccatae; and often a note on the economic import of the species is added.

In the sequence of families and in nomenclature the work accords with the seventh edition of GRAY'S *Manual*. A statistical summary gives the following composition of the flora: number of families 134, genera 621, species 1942, varieties and forms 286; a total of 2228 recognizably distinct plants; and approximately four-fifths of these are indigenous to the state.

The work is an important one to the taxonomic student; and the collaborators have done a commendable service to their state in recording, in available and useful form and with a high degree of accuracy and completeness, their intimate knowledge of the Connecticut flora.—J. M. GREENMAN.

North American Flora.[6]—Volume XXV, part II, is devoted to the Geraniales, as follows: Tropaeolaceae by G. V. NASH, Balsaminaceae and Limnanthaceae by P. A. RYDBERG, Koeberliniaceae by J. H. BARNHART, Zygophyllaceae by A. M. VAIL and P. A. RYDBERG, and the Malpighiaceae by J. K. SMALL. Four new genera of the Malpighiaceae are proposed, namely, *Adenoporces*, *Callaeum*, *Rosanthus*, and *Banisteriopsis*. Several new species are described; these are distributed among the following genera: *Impatiens* (3), *Fagonia* (3), *Guaiacum* (3), *Kallstroemia* (6), *Mascagnia* (2), *Hiraea* (1), *Triopteris* (1), *Tetrapteris* (2), *Banisteriopsis* (2), *Banisteria* (2), *Stigmaphyllon* (1), *Thryallis* (1), and *Malpighia* (5).—J. M. GREENMAN.

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[5] GRAVES, C. B., EAMES, E. H., BISSELL, C. H., ANDREWS, L., HARGER, E. B., and WEATHERBY, C. A., Committee of the Connecticut Botanical Society. Catalogue of the flowering plants and ferns of Connecticut growing without cultivation. Bulletin No. 14, State Geological and Natural History Survey. 8vo. pp. 569. Hartford, Conn. 1910.

[6] North American Flora, vol. XXV, part II, pp. 89–171. New York Botanical Garden. 1910.

NOTES FOR STUDENTS

Current taxonomic literature.—L. ABRAMS (Bull. Torr. Bot. Club **37**:149–153. 1910) has described 4 new species of flowering plants from California.—O. AMES (Phil. Journ. Sci. Bot. **4**:663–676. 1909) in continuation of his studies on Philippine orchids records 30 species, 11 of which are new to science.—S. F. BLAKE (Fern Bull. **18**:9, 10. 1910) records a new variety of *Lycopodium tristachyum* Pursh from New Hampshire.—I. BOLDINGH (Recueil Trav. Bot. Néerl. **6**:1–36. 1909) under the title "A contribution to the knowledge of the flora of Anguilla (B.W.I.)" lists about 150 species of flowering plants. The list is based on collections made on the island by Mr. BOLDINGH in September of 1906.—F. BØRGESEN (Bot. Tidssk. **30**:1–19. *pls. 1, 2.* 1910) has published the results of his studies in the Florideae collected in the waters of the Danish West Indies, describing and illustrating 5 new species.—M. BURRET (Bot. Jahrb. **44**:198–238. 1910) presents the results of an extended taxonomic study of the genus *Grewia*, accompanying the same by a key to the African species.—B. CHABAUD (Rev. Hort. Paris **82**:58–60. *fig. 18.* 1910) has published a new species of palm (*Sabal uresana*) indigenous in the state of Sonora, Mexico.—H. CHRIST (Rep. Nov. Sp. **8**:17–20. 1910) has published 7 new species of ferns from Costa Rica.—A. COGNIAUX, A. LINGELSHEIM, F. PAX, and H. WINKLER (*ibid.* 1–6) under the title "Plantae novae bolivianae IV" have published 11 new species and 2 new varieties of Bolivian flowering plants.—A. ENGLER (Bot. Jahrb. **44**:137–155. 1910) has published 31 new species of the Burseraceae from Africa; the same author (*ibid.* Beibl. 101, p. 34) proposes a new genus (*Scirpodendron*) of the Cyperaceae from Africa.—E. GILG (*ibid.* 15–19) describes and illustrates a new species of *Marckea* (*M. Peckoltiorum*) from Brazil.—W. FAWCETT and A. B. RENDLE (Journ. Bot. **48**:106–108. 1910) in continuation of their studies on the orchids of Jamaica have published 4 new species.—E. L. GREENE (Leafl. Bot. Obs. and Crit. **2**:25–88. 1910) has characterized about 90 new species of flowering plants mostly from western United States.—E. HASSLER (Bull. Soc. Bot. Genève II. **2**:32–36. 1910) has characterized 3 varieties and 6 forms of *Indigofera campestris* Bong. from Paraguay. The same author (Rep. Nov. Sp. **8**:28–31, 34–47, 66–73, 113–132, 204–210. 1910), in cooperation with different specialists, has published several new species and varieties of flowering plants from South America. Two new genera of the Malvaceae are proposed, namely *Blanchetiastrum* from Brazil, and *Bastardiopsis* from Brazil and Paraguay.—A. VON HAYEK (Oesterr. Bot. Zeitschr. **60**:89–93. 1910) has characterized a new genus (*Degenia*) of the Cruciferae, based on *Lesquerella velebitica* Degen.—A. A. HELLER (Muhlenbergia **6**:13–32. 1910) records the results of further studies on the "Nevada lupines" and includes descriptions of 4 new species.—R. H. HOWE, Jr. (Bull. Torr. Bot. Club **37**:1–18. *pls. 1–7.* 1910) gives a revision of the genus *Usnea*, as represented in North America north of the 15th parallel, recognizing 4 species.—E. KOEHNE (Rep. Nov. Sp. **8**:16, 17, 165–167, 196–199. 1910) has published 6 new species and 4 new varieties of *Cuphea* from the Lesser Antilles and Paraguay.—F.

KRÄNZLIN (*ibid.* 97, 98) has described 2 new species of orchids from the Philippine Islands.—G. KÜKENTHAL (*ibid.* 7, 8) under the heading "Cyperaceae novae I" describes new species and varieties in the genus *Carex*, some of which are from America.—K. KRAUSE (Bot. Jahrb. **44**: Beibl. 101, pp. 9–14. 1910) has published several new species of Araceae from South America and the Philippine Islands.—TH. LOESENER (*ibid.* **44**: 156–197) gives a synoptical revision of the genus *Salacia*, recognizing 64 species, of which 25 are new to science.—W. MOESER (*ibid.* 239–345) characterizes the different natural groups of species in the genus *Helichrysum*, accompanying the same by determinative keys, and adding about 30 species and several varieties new to science.—R. PILGER (*ibid.* Beibl. 101, p. 7) has published a new species of *Valeriana* from southern Brazil.—J. W. PALIBINE (Bull. Soc. Bot. Genève II. **2**: 17–21. 1910) presents an article on the subsection BAICALIA of the genus *Oxytropis*, describing one new species and transferring *Arogallus Bellii* Greene to *Oxytropis*.—F. PAX (Rep. Nov. Sp. **8**: 161, 162. 1910) has published 4 new species of Euphorbiaceae from Brazil and Mexico.—H. E. PETERSEN (Bot. Tidssk. **29**: 345–429. 1909) under the title "Studier over Ferskvands-Phykomyceter" describes several new species and proposes one new genus (*Pythiomorpha*) from Denmark.—A. PULLE (Recueil Trav. Bot. Néerl. **6**: 251–293. 1909) in continuation of his studies on the flora of Surinam publishes a second article including 18 new species of flowering plants.—C. B. ROBINSON (Phil. Journ. Sci. Bot. **4**: 687–698. 1909) gives a synoptical treatment of the Philippine Boraginaceae, recognizing 10 genera to which are referred 21 species.—P. A. RYDBERG (Bull. Torr. Bot. Club **37**: 127–148. 1910) under "Studies on the Rocky Mountain flora XXI" describes 27 new species and makes several new combinations in the Compositae.—F. J. SEAVER (Mycologia **2**: 48–92. *pls. 20, 21.* 1910) continues his monographic treatment of the Hypocreales of North America, publishes several new species, and proposes two new genera (*Chromocrea* and *Chromocreopsis*).—H. and P. SYDOW (Ann. Mycol. **8**: 36–41. 1910) have published 22 new species of fungi from the Philippine Islands.—W. TRANZSCHEL (*ibid.* 1–35) in an article entitled "Die auf der Gattung Euphorbia auftretenden autöcischen Uromyces-Arten" has published several new species of fungi parasitic on different American euphorbias.—P. WILSON (Bull. Torr. Bot. Club **37**: 85, 86. 1910) describes a new species of *Amyris* from Mexico and publishes several new combinations in the genus *Zanthoxylum*.—E. O. WOOTON (*ibid.* 31–41) in an article entitled "The larkspurs of New Mexico" recognizes 12 species for the state, 5 of which are described as new; a key to the species accompanies the descriptive matter.—Different authors (Kew Bull. 55–60. 1910) under the title "Diagnoses Africanae XXXIV" have published several new species and described a new genus (*Megabaria*) of the Euphorbiaceae.—J. M. GREENMAN.

Respiration.—KUIJPER[7] has studied the effect of various temperatures on the amount of CO_2 produced by the seedlings of *Lupinus luteus*, *Pisum sativum*,

[7] KUIJPER, J., Ueber den Einfluss der Temperatur auf die Atmung der höheren Pflanzen. Extrait Rec. Trav. Bot. Néerland. **7**: pp. 109. *pls. 3.* 1910.

and *Triticum vulgare*, with the view of testing the applicability of the ideas set forth in BLACKMAN'S[8] "optima and limiting factors" to the process of respiration. In general the experiments were run six hours, and the CO_2 production determined for each hour. Up to 10° the CO_2 produced per hour was constant for the six hours. At higher temperatures, up to 20°, there was a rise for four or five hours. This rise is hard to explain in the light of the facts that the seeds were germinated at approximately 20°, that it occurred regardless of the age of the seedlings, and that it was followed by a corresponding fall. At constant temperatures above this, up to 40°, a fluctuation in CO_2 production was apparent. Especially between 30° and 40° this manifested itself by a rapid fall for the first two hours, followed by a later rise ranging over one to two hours, followed by a later continuous fall. The author assumes that two distinct processes are differently affected by the continuous high temperature: one early depressed, marking the fall; the other later stimulated, showing the rise. This fact he suggests may be related to the double nature of CO_2 production in respiration noted by PALLADIN, in which he assumes the action of oxidase on the one hand and of carbonase on the other. At still higher temperatures there is a continual fall in CO_2 production. The temperature at which one type of behavior changes to another is determined by the nature of the stored food, as the following tables show:

	Lupinus	Pisum	Triticum
Rise noticeable	15–20°	20–25°	30°
Fluctuation	20–25°	30°	35°
Continual falling	25°	35°	40°
Protein	37%	22%	12%
Starch	none	54%	74%

The VAN'T HOFF law applies for *Pisum* and *Triticum* from 0°–20°, and for *Lupinus* up to 25°. The coefficient for a temperature difference of 10° lies between 2 and 3. The continual falling in CO_2 production with continual exposures at higher temperatures agrees with BLACKMAN'S results. BLACKMAN found that in photosynthesis the initial rate at any given high temperature (30–40°) could be figured in two ways, giving agreeing results: by applying the VAN'T HOFF coefficient to the measurements at lower temperatures where the rate is constant, or by taking several later determinations at the given temperatures and from these extrapolating the initial value. KUIJPER finds that these methods will not apply to the CO_2 yield in respiration, because of the appearance of the two antagonistic factors at temperatures between 30° and 40°, and because of the non-application of the VAN'T HOFF law at the higher temperatures.—WILLIAM CROCKER.

Permeability.—CZAPEK[9] has published a preliminary article upon the effect of various reagents upon the precipitation of tannins in plant cells by means of caffein and ammonium carbonate. If slices from the epidermis of *Echeveria*

[8] BLACKMAN, F. F., Annals of Botany **19**:281–295. 1905.

[9] CZAPEK, F., Versuchs über Exosmose aus Pflanzenzellen. Ber. Deutsch. Bot. Gesell. **28**:159–169. 1910.

leaves are placed in $n/1000$ acid for 24 hours, and then treated with caffein solution, no tannin is precipitated; while the controls kept in water show a heavy precipitate. The acid solution showed a strong tannin test with iron salts. CZAPEK concludes that the acid renders the *Plasmahaut* permeable to tannins, and that they gradually diffuse out. In strong acids he finds $n/6400$ the critical (minimum) concentration for inducing marked permeability. This is the concentration at which KAHLENBERG and TRUE found growth to cease. CZAPEK believes the stoppage of growth is directly due to reduced turgor, resulting from the induced permeability of the protoplasm to contained substances. He recognizes that injuries appear in concentrations below the critical concentration for permeability. Various other substances give similar results. The critical concentration for phenol is 0.58 per cent or $n/16$; for resorcin, di-hydric phenol, 2.85 per cent or $n/4$; for pyrogallol tri-hydric phenol $n/4$. Among alcohols, the critical concentrations were methyl 15 per cent, ethyl 10–11 per cent, normal and iso-propyl 4–5 per cent, iso-butyl 1–2 per cent, amyl 0.5 per cent. In acetic acid the critical concentration is below that determined by its acid properties. The effect of external conditions upon the permeability of protoplasmic and other plant membranes is a subject that deserves much attention from plant physiologists. Animal physiologists are teaching us much in this field.—WILLIAM CROCKER.

Morphology of Sciadopitys.—LAWSON[10] has published an account of the gametophytes and embryo of *Sciadopitys*, one of the peculiar conifers of eastern Asia. The microspores are binucleate at shedding, and are received upon the so-called pollen cushion, which is the tip of the nucellus differentiated into a loose tissue of large thin-walled cells. During the first season only one more division occurs, that of the generative cell into the body cell and a free stalk nucleus. In the next season the body cell passes toward the tip of the pollen tube, which has entered an archegonial chamber, and there produces two unequal male nuclei. The formation of the megaspore tetrad is peculiar. The division of the mother cell nucleus is not accompanied by wall formation; but the subsequent division of the two daughter nuclei is; so that the tetrad comprises three cells, the middle one of which is binucleate. In these divisions the chromosome numbers prove to be 8 and 16. The innermost megaspore is the functioning one, enlarging very much, and becoming invested by the usual zone of nutritive cells. The female gametophyte is developed as in the majority of conifers, and there are four or six archegonia, each with its own investment of jacket cells and a deep archegonial chamber. A ventral nucleus is formed, but no ventral canal cell. In the development of the embryo, the free nuclei pass to the base of the egg at the four-nucleate stage, and the proembryo finally consists of three tiers of cells and one tier of free nuclei toward the egg cytoplasm. The middle tier of cells becomes the suspensor, while the embryo-forming tier becomes a mass of at least sixteen cells.—J. M. C.

[10] LAWSON, A. ANSTRUTHER, The gametophytes and embryo of *Sciadopitys verticillata*. Annals of Botany **24**:403–421. *pls. 29–31*. 1910.

The structure of Podocarpus spinulosa.—BROOKS and STILES[11] find the structure of the stem and leaf of *Podocarpus spinulosa* similar to species studied by PENHALLOW and WORSDELL. The wall of the microsporangium is described as similar to that of *Saxegothaea* and *Araucaria* except that the dehiscence is oblique. The male gametophyte agrees with *P. ferruginea* and *P. dacrydioides*, described by JEFFREY and CHRYSLER, having a prothallial complex of eight cells, and occasionally the appearance as of a second derivative of the generative cell. The course of the vascular bundles in the ovulate sporophyll is studied in detail and compared with other forms.

That *Podocarpus* is a specialized offshoot from *Saxegothaea*-like ancestors is confirmed by the presence of less mesarch wood than in *Saxegothaea*, the loss of function of some of the resin canals, and the specialized ovulate structure with reduction in size and number of scales. The independence of the vascular supply of the ovule from that of the scale is explained by the greater importance of the ovule in *Podocarpus*. The authors regard Podocarpineae as a natural group, with no very definite connection with Taxineae, no evident relation to Abietineae, but with a probable connection with Araucarineae.—MARY S. YOUNG.

Evolution of plants.—In his presidential address of 1910 before the Linnean Society of London, PROFESSOR SCOTT selected as his subject "Some modern ideas on the course of evolution of plants." It is an outline of the present status of opinion in reference to the evolution of vascular plants, especially as developed by the recent rapid increase of knowledge of paleobotanical material, and is in part a confession of faith. The author evidently believes in the homologous origin of the alternation of generations, and regards the sporophyte of the pteridophyte as developed directly from the thallophyte body. Special attention is given to the views of LIGNIER in connection with this thalloid origin of the cormophyte. The classification of vascular plants proposed in the new edition of his *Studies in fossil botany* is outlined, and the gymnosperm relationships are discussed; while Bennettitales-like forms are still put forward as representing a possible origin of the angiosperms. Perhaps the main thesis of the address is to illustrate among plants the theory of GASKELL, developed in a discussion of the origin of vertebrates, that "each successive group has arisen from some member of the highest group existing at the time."—J. M. C.

Evolution of Pinus.—BAILEY[12] has presented the anatomical characters that seem to be of service in tracing the lines of descent of pines. The cretaceous pines and *Prepinus* are characterized by thick-walled ray parenchyma, "piciform" lateral ray pits, absence of marginal ray tracheids, and abundant

[11] BROOKS, M. A., and STILES, W., The structure of *Podocarp spinulosa*. Annals of Botany **24**:305–318. *pl. 21*. 1910.

[12] BAILEY, IRVING W., Anatomical characters in the evolution of *Pinus*. Amer. Nat. **44**:284–293. 1910.

tangential pitting of autumnal tracheids. The change in the living pines is seen in the disappearance of thick-walled ray cells, the presence of large compound ray pits, the development of ray tracheids, and the loss of tangential pitting of autumnal tracheids. The type of hard pines represented by *P. resinosa* in North America and *P. silvestris* in Europe is "the most highly developed and specialized condition among living pines." The nut pines (of North America and Asia) have piciform lateral ray pits and thick-walled ray cells, and in these features they are the living pines that approach most nearly to the cretaceous pines. The hard pines of the United States, with the exception of *P. resinosa*, show a great range of variation from piciform to compound lateral ray pits; and the soft pines present a parallel series of gradations.—J. M. C.

Non-available water.—BOVIE[13] has tested the effect of salts upon the non-available water in a soil of crushed quartz. Aside from a full nutrient solution of 0.2 per cent, various amounts of NaCl, ranging from 0.05 to 0.6 per cent, were added. To 100 grams of soil 20 grams of solutions were added. After the plants had grown considerably and the soil moisture was nearly exhausted, the cultures were placed in a special drying chamber of relative humidity of 0.1. Soil was tested for contained moisture when the foliage began to wilt, and when it showed drying. The remaining water was essentially the same regardless of the amount of salt present. Assuming that none of the salts are absorbed by the plants, BOVIE finds that the soil water, at the close of the experiments, in some cases would contain more than 300 per cent of salts, and that much of it must be in the solid form in spite of greatly increased solubility in the thin water films. He also offers some evidence for the movement of water in soils of low water content in the form of vapor, a thing already emphasized by various workers.—WILLIAM CROCKER.

Transpiration stream.—ZIJLSTRA[14] finds that lowering the temperature of sections (20 cm. in length) of stem of intact plants to 0° C. for several days, even under the most favorable conditions for transpiration, does not lead to wilting of the foliage. It is assumed that this renders the living cells of the zone comparatively inactive, without injuring them and without producing injurious or blocking material. The results are contrary to those obtained by the same method by URSPRUNG, who used the results as an argument for the necessity of the activity of living cells to the continuity of the transpiration stream. ZIJLSTRA also conducted a number of experiments on the movement of 0.1 per cent *Säureviolett* (GRÜBLER) in living and dead stems. He also

[13] BOVIE, WILLIAM T., The effects of adding salts to the soil on the amount of non-available water. Bull. Torr. Bot. Club **37**:273–292. 1910.

[14] ZIJLSTRA, K., Contributions to the knowledge of the movement of water in plants. Reprint from Koninklijke Akad. Wetenschappen te Amsterdam. 1910:574–584.

studied the behavior of plants in which lateral movement of water was made necessary by the ordinary method of incisions on opposite sides. Only the first set of experiments throws any new light on the question of the rise of sap.—WILLIAM CROCKER.

Phylogeny of Filicales.—BOWER[15] has begun the publication of a series of studies in the phylogeny of the Filicales, and the first paper presents the singular and poorly known genus *Plagiogyria*. It is grouped among the Polypodiaceae, and even merged with *Lomaria*, but the position is anomalous on account of the oblique annulus. Several species were secured, both oriental and occidental, and the results may be considered fairly representative for a genus of eleven species. The conclusions are that the genus is quite a distinct one, deserving to stand apart from *Lomaria* or any other genus; that it is relatively primitive, as shown by stelar structure, leaf traces, venation, etc.; that it has resemblances to the Simplices, but that its characters indicate that it is rightly placed among the Pterideae; and that its "mixed" sorus, without any "gradate" sequence of sporangia, combined with its primitive characters and its affinity to the Pterideae, shows that the Mixtae have probably come directly from the Simplices, rather than by any of the Gradatae.—J. M. C.

Geotropism and split stems.—SCHTSCHERBACK,[16] working in PFEFFER'S laboratory, has carried on a series of experiments on the geotropic reactions of split stems. In the main, *Lupinus albus* was used, for in this form the tissue strains between cortex and pith are slight. Splitting cuts the rate of growth even in the vertical position, but two equal halves grow with equal speed in that position. If the two halves are placed horizontally, with the split faces together and horizontal, the lower half is favored in its growth and the upper half greatly inhibited, or even entirely stopped in most cases. Restoring the two halves to their normal vertical position restores them to equal growth, and rotating them 180° on the horizontal axis, to give an interchange of position, gives an interchange of rates of growth. The same geotropic inhibition and favoring of growth occurs in the two separated halves of the stem as occur in their intact condition.—WILLIAM CROCKER.

Relation of living cells to the rise of sap.—RORHARDT,[17] in his voluminous article on the part played by living cells in the rise of sap, has followed the method of wearying the reader with the publication of his notebook, instead of preparing a concise statement of his methods and results. Of the 125 different species of herbs and shrubs native to Germany, representing various monocotyl and dicotyl

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[15] BOWER, F. O., Studies in the phylogeny of the Filicales. I. *Plagiogyria*. Annals of Botany **24**:423–450. *pls. 32, 33. figs. 5.* 1910.

[16] SCHTSCHERBACK, JOHANN, Die geotropische Reaktion in gespaltenen Stengeln. Beih. Bot. Centralbl. **25**[1]:358–386. 1910.

[17] RORHARDT, P. A., Ueber die Beteiligung lebender Zellen am Saftsteigen bei Pflanzen von niedrigen Wuchs. Beih. Bot. Centralbl. **25**[1]:243–357. *figs. 2.* 1910.

families that he has investigated, he finds that the killing of a short zone of a stem or of a petiole results very soon in cutting down the water transport to the parts above, and leads to wilting—the longer the killed zone, the quicker the wilting. He claims that there is no blocking or interfering with the water path, but that the reduced water movement is due to the lack of living cells in the zone killed. This indicates that the continuous rise of sap in small plants, as has been shown to be the case in tall trees, is dependent upon the action of living cells.—WILLIAM CROCKER.

Seedling structure of Gnetales.—HILL and DEFRAINE[18] conclude their account of the seedling structure of gymnosperms by a presentation of Gnetales. A short cotyledonary tube is formed in every case; the cotyledonary traces are two in *Ephedra*, four (in two pairs) in *Welwitschia*, and four or five in *Gnetum;* and all the traces are collateral endarch. The so-called foot in *Welwitschia* and *Gnetum* is described as a parenchymatous growth at the base of the hypocotyl, in the former genus being spadelike and with no vascular supply, and in the latter genus being rodlike and with numerous well-differentiated bundles. The transition to root structure occurs in the lower region of the hypocotyl, in *Welwitschia* and *Gnetum*, immediately below the foot; and in all cases the primary root is diarch.—J. M. C.

"Apogamy" in Pteris.—Miss STEPHENS and Miss SYKES have examined prothallia of *Pteris droogmantiana* furnished by BOODLE. In a brief note[19] they announce that binucleate cells are common; that there has been no nuclear migration, as no surrounding cell is without its nucleus; that the two nuclei arise from the division of the nucleus of an ordinary cell without wall-formation; that the two nuclei thus produced remain separate for some time and then fuse. Just what this performance means is not evident, but perhaps the fuller paper will tell. The note is called one on "apogamy," and presumably there is reason for knowing that these are apogamous prothallia.—J. M. C.

Morphology of Psilotum.—STILES[20] has investigated the anatomical structure of the serial shoots of *Psilotum flaccidum*, one of the two species usually regarded as constituting the genus. Aside from certain anatomical details that distinguish this species from the other (*P. triquetrum*), the main results are that secondary thickening is found in this species also; that mesarch structure occurs in the lower part of the aerial stems; that the sporangiophore trace terminates in the central tissue between the three "confluent" sporangia, as had been shown for *Tmesipteris;* and that no evidence was obtained to decide whether the sporangiophore is "foliar" or is "an organ *sui generis*."—J. M. C.

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[18] HILL, T. G., and DEFRAINE, E., On the seedling structure of gymnosperms. IV. Gnetales. Annals of Botany **24**:319–333. *pls. 22, 23.* 1910.

[19] STEPHENS, E. L., and SYKES, M. G., Preliminary note on apogamy in *Pteris droogmantiana.* Annals of Botany **24**:487. 1910.

[20] STILES, W., The structure of the aerial shoots of *Psilotum flaccidum* Wall. Annals of Botany **24**:373–397. *pl. 25.* 1910.

Removal of cotyledons.—JACOBI[21] has studied the effect of the removal of various amounts of cotyledonary tissue upon the rate of growth of seedlings. In both light and darkness, removal of one cotyledon and even a part of the second early but temporarily stimulates the rate of longitudinal growth of the stem of *Phaseolus multiflorus*. In *Cucurbita Pepo* and conifer seedlings, elongation of the stem is stimulated in darkness, while in light the expanse of the cotyledon is most increased. In *Cucurbita* and the pines, the cotyledons of course bear little food material and function as leaves. The paper adds little that is new.—WILLIAM CROCKER.

Morphology of Cunninghamia.—In 1908 MIYAKE published a preliminary account of the gametophytes and embryo of the monotypic *Cunninghamia*, which was reviewed in this journal (**46**:156. 1908). Now the full paper has appeared,[22] with its excellent plates and photomicrographs. The review referred to includes a synopsis of the most important results, so that it is only necessary to announce the appearance of the full paper.—J. M. C.

Insect galls.—BEUTENMULLER[23] has published two more valuable papers on the American gall-producing insects and their galls. In these two papers he takes up 44 species, including three new ones, gives complete synonomy and bibliography for each, and clear, comprehensive descriptions of both the galls and the insects. This series of papers is of very great value to both the botanist and the entomologist.—MEL T. COOK.

A new disease of apples.—LEWIS[24] has recently described a new species of *Endomyces* (*E. mali*), which he finds capable of producing a slow decay of ripe apples. This is the first species of this family to be reported from the United States, and hence many will be interested in the facts he gives regarding the morphology, spore production, relationship, and cultural behavior of this new species. —E. MEAD WILCOX.

Polarity.—Miss FREUND[25] has made a study of polarity in the twigs of plants. The paper offers nothing new on the subject. Her work might have been far more conclusive if she had been acquainted with the work of MACALLUM,[26] and had applied the cultural methods used by him.—WILLIAM CROCKER.

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[21] JACOBI, HELENE, Ueber den Einfluss der Verletzung von Kotyledonen auf das Wachstum von Keimungen. Flora **101**:279–289. *figs. 2*. 1910.

[22] MIYAKE, K., The development of the gametophytes and embryogeny in *Cunninghamia sinensis*. Beih. Bot. Centralbl. **27**:1–25. *pls. 1–5*. 1910.

[23] BEUTENMULLER, WM., The North American species of *Neuroterus* and their galls. Amer. Mus. Nat. Hist. **28**:117–136. *pls. 8–13*. 1910; and the North American species of *Aylax* and their galls. *Idem* **28**:137–144. *pl. 14*. 1910.

[24] LEWIS, C. E., An *Endomyces* from apple. Bull. Maine Exp. Sta. **178**:45–64. *figs. 58–71*. 1910.

[25] FREUND, YELLA, Untersuchungen über Polarität bei Pflanzen. Flora **1**:279–308. 1900.

[26] MACALLUM, W. B., Regeneration in plants. BOT. GAZETTE **40**:97–120, 241–263. 1905.